CERTIFICATE OF MAILING BY "EXPRESS MAIL"

In re: U.S. Patent No. 6,444,673 Issue date: September 3, 2002

Patentee: Claude COTREL, *et al.* Assignee: Sepracor Inc.

Title: OPTICALLY ACTIVE 5H-PYRROLO[3,4-B] PYRAZINE DERIVATIVE, ITS PREPARATION AND PHARMACUETICAL COMPOSITIONS CONTAINING IT

Attorney Docket No.: 0701.243

"EXPRESS MAIL" Mailing Label No.: **EV 582715141 US**

Date of Deposit: February 11, 2005

Enclosed are:

- Express Mail Certificate for Label No.: EV 582715141 US
- One (1) Acknowledgment Postcard
- Request for Extension of Patent Term Under 35 U.S.C. §156 (15 pps) (1 original and 2 copies)
- Exhibit A (6 pps) and Exhibit B (29 pps) (in triplicate)
- Check for $1,120.00 (Extension Fee)

37 CFR 1.10 Certification

I hereby certify that this paper and the indicated enclosures are being deposited with the U.S. Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and addressed to:

Mail Stop Patent Ext.
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

CHRISTINE C. WEBER
(Typed or printed name of person mailing paper or fee)

(Signature of person mailing paper or fee)

09/937,135 02-14-05 FAC

BOX PATENT EXT.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Atty. Docket No. 0701.243

In re: U.S. Patent No. 6,444,673

Patentee: Claude COTREL, *et al.*

Assignee: Sepracor Inc.

Issue date: September 3, 2002

REQUEST FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. §156

Director of the United States Patent and Trademark Office

Washington, D.C. 20231

BOX PATENT EXT.

Sir:

Pursuant to Section 201(a) of the Drug Price Competition and Patent Term Restoration Act of 1984, 35 U.S.C. § 156, Sepracor Inc. ("Sepracor"), represents that it is the owner of record of United States Patent No. 6,444,673 and hereby requests an extension of the patent term of U.S. Patent No. 6,444,673.

The following information is submitted in accordance with 35 U.S.C. § 156(d) and 37 C.F.R. § 1.740, and follows the format and requirements set forth in 37 C.F.R. § 1.740.

(1) "A complete identification of the approved product as by appropriate chemical and generic name, physical structure or characteristics;" 37 C.F.R. §1.740(a) (1).

The approved[1] product is LUNESTA™ (eszopiclone), film-coated tablets, 1 mg, 2 mg and 3 mg, for oral administration. The generic name for the approved product is eszopiclone, which is indicated for the treatment of insomnia, including difficulty falling asleep and/or maintaining sleep.

Synonyms for eszopiclone are:

ESTORRA™;

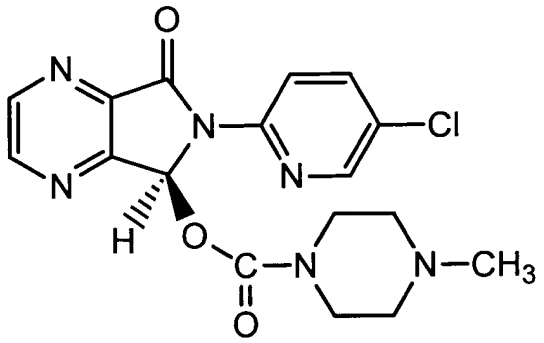
esopiclone;

(+)-zopiclone; and

(S)-zopiclone.

The eszopiclone is identified by the following:

(a) Structural Formula:



(b) Chemical names:

[1] As described more fully on pages 3-4, this request for extension is submitted based on FDA's determination that LUNESTA™ was "approved" on December 15, 2004, for purposes of determining patent term restoration. Sepracor questions this determination by FDA and does not waive its right to challenge that agency determination following submission of this request for extension.

(+)-(5S)- 6-(5-chloro-2-pyridyl)-5-[(4-methyl-1-piperazinyl)carbonyloxy]-7-oxo-6,7-dihydro-5H-pyrrolo[3,4-b]pyrazine;

(+)-(5S)-6-(5-chloropyridin-2-yl)-7-oxo-6,7-dihydro-5H-pyrrolo[3,4-b]pyrazin-5-yl 4-methylpiperazine-1-carboxylate; and

1-Piperazinecarboxylic acid, 4-methyl-, (5S)-6-(5-chloro-2-pyridinyl)-6,7-dihydro-7-oxo-5H-pyrrolo[3,4-b]pyrazin-5-yl ester.

(c) Molecular Weight: 388.81

(d) Empirical Formula: $C_{17}H_{17}ClN_6O_3$

(2) "A complete identification of the Federal statute including the applicable provision of law under which the regulatory review occurred;" 37 C.F.R. § 1.740(a)(2).

Section 505 of the Federal Food, Drug, and Cosmetic Act (FDC Act), 21 U.S.C. § 355, is the Federal statute under which the Food and. Drug Administration's (FDA's) regulatory review of Sepracor's LUNESTA™ investigational new drug (IND) application and new drug application (NDA) for eszopiclone occurred. Section 505(b) of the FDC Act, 21 U.S.C. § 355(b), authorizes the filing of an NDA for a "new drug". The FDA subsequently approved the LUNESTA™ NDA (021-476) under the authority granted the agency by Section 505(c) of the FDC Act, 21 U.S.C. § 355(c).

(3) "An identification of the date on which the product received permission for commercial marketing or use under the provision of law under which the applicable regulatory review period occurred;" 37 C.F.R. § 1.740(a)(3).

On December 15, 2004, the FDA issued an "approval letter" for Sepracor's LUNESTA™ (eszopiclone) NDA under Section 505 of the FDC Act. Although FDA takes the position

that the product was "approved" on that date for purposes of determining patent term extension, Sepracor questions whether "the product received permission for commercial marketing" on that date within the meaning of the statute. Sepracor notes that FDA has taken the position that Sepracor cannot market LUNESTA™ under the terms of the "approval letter" until the product is listed on the Schedule of Controlled Substances under 21 USC §823. Nonetheless, in order to avoid potential forfeiture under 35 USC §156(d)(1) based on FDA's current interpretation of the statute, Sepracor submits this request for extension based on FDA's determination that LUNESTA™ was "approved" for purposes of patent term extension on December 15, 2004. In so doing, Sepracor does not waive its right to challenge that determination by FDA.

(4) "In the case of a drug product, an identification of each active ingredient in the product and as to each active ingredient, a statement that it has not been previously approved for commercial marketing or use under the Federal Food, Drug and Cosmetic Act, the Public Health Service Act, or the Virus-Serum- Toxin Act, or a statement of when the active ingredient was approved for commercial marketing or use (either alone or in combination with other active ingredients), the use for which it was approved, and the provision of law under which it was approved." 37 C.F.R. § 1.740(a)(4).

The active ingredient in LUNESTA™ (eszopiclone) film-coated tablets is eszopiclone. Eszopiclone has not been previously approved for commercial marketing or use under the Federal Food, Drug and Cosmetic Act, the Public Health Service Act or the Virus-Serum-Toxin Act. Eszopiclone is the dextrotatory enantiomer of the racemic mixture zopiclone. Zopiclone has not been previously approved by the FDA.

(5) "A statement that the application is being submitted within the sixty day period permitted for submission pursuant to § 1.720(f) and an identification of the last day on which the application could be submitted;" 37 C.F.R. § 1.740(a)(5)

This application is being submitted within the sixty-day period following FDA approval of the LUNESTA™ (eszopiclone) NDA. FDA approved the LUNESTA™ (eszopiclone) NDA on December 15, 2004. The sixty-day period following approval of the NDA for submission of this patent extension application will expire on February 13, 2005.

(6) "A complete identification of the patent for which an extension is being sought by the name of the inventor, the patent number, the date of issue, and the date of expiration;" 37 C.F.R. § 1.740(a)(6).

U.S. Patent No. 6,444,673

Inventors: Claude COTREL and Gerard ROUSSEL

Issue date: September 3, 2002

Expiration Date: January 16, 2012

(7) "A copy of the patent for which an extension is being sought, including the entire specification (including claims) and drawings;" 37 C.F.R. § 1.740(a)(7).

A copy of U.S. Patent No. 6,444,673 is attached as Exhibit A.

(8) "A copy of any disclaimer, certificate of correction, receipt of maintenance fee payment, or re-examination certificate issued in the patent," 37 C.F.R. § 1.740(a)(8).

U.S. Patent No. 6,444,673 issued on September 3, 2002. Maintenance fees are not due until March 3, 2006.

No disclaimer, certificate of correction or re-examination certificate has issued to date in connection with U.S. Patent No. 6,444,673.

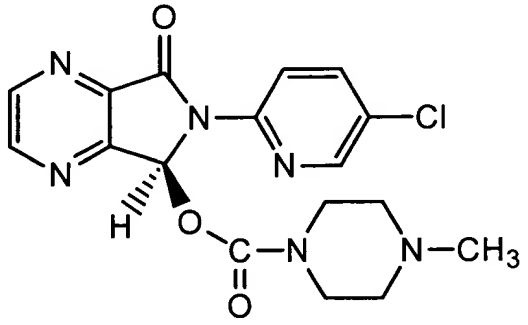
(9) "A statement that the patent claims the approved product or a method of using or manufacturing the approved product, and a showing which lists each applicable patent claim and demonstrates the manner in which one such patent claim reads on:

(i) The approved product if the listed claims include any claim to the approved product;

(ii) The method of using the approved product, if the listed claims include any claim to the method of using the approved product;

(iii) The method of manufacturing the approved product, if the listed claims include any claim to the method of manufacturing the approved product;" 37 C.F.R. §1.740(a)(9).

U.S. Patent No. 6,444,673 claims the approved product eszopiclone. Claims 1, 3 and 4 are directed to the dextrorotatory isomer of 6-(5-chloro-2-pyridyl)-5-[(4-methyl-1-piperazinyl)carbonyloxy]-7-oxo-6,7-dihydro-5H-pyrrolo[3,4-b]pyrazine and pharmaceutically acceptable salts thereof. Claims 2, and 5-8 are directed to a pharmaceutical composition comprising eszopiclone. Eszopiclone is the generic name for the dextrorotatory isomer of 6-(5-chloro-2-pyridyl)-5-[(4-methyl-1-piperazinyl)carbonyloxy]-7-oxo-6,7-dihydro--5H-pyrrolo[3,4-b]pyrazine and has the following formula:



Representative claims of U.S. Patent No. 6,444,673 are reproduced below.

1. 6-(5-chloro-2-pyridyl)-5-[(4-methyl-1-piperazinyl)carbonyloxy]-7-oxo-6,7-dihydro-5H-pyrrolo[3,4-b]pyrazine, or a pharmaceutically acceptable salt thereof, in the form of its dextrorotatory isomer and essentially free of its levorotatory isomer.

2. A pharmaceutical composition comprising an effective amount of the dextrorotatory isomer, essentially free of the levorotatory isomer of 6-(5-chloro-2-pyridyl)-5-[(4-methyl-1-piperazinyl)-carbonyloxy]-7-oxo-6,7- dihydro-5H-pyrrolo[3,4-b]pyrazine, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

(10) A statement, beginning on a new page of the relevant dates and information pursuant to 35 U.S.C. § 156(g) in order to enable the Secretary of Health and Human Services, or the Secretary of Agriculture, as appropriate, to determine the applicable regulatory review period as follows:

(i) For a patent claiming a human drug, antibiotic or human biological product:

(A) The effective date of the investigational new drug (IND) application and the IND number;

(B) The date on which a new drug application (NDA) ....was initially submitted and the NDA or PLA number :and

(C) The date on which the NDA was approved or the Product License issued; 37 C.F.R. § 1.740(a)(10)(i).

In order to enable the Secretary to determine the applicable regulatory review period, the following information is provided:

(a) Sepracor Inc. filed its Investigational New Drug (IND) application for LUNESTA™ (eszopiclone) on July 22, 1999 (IND 58,647), and it became effective on August 21, 1999;

(b) Sepracor Inc. initially submitted a new drug application (NDA) for LUNESTA™ (eszopiclone) to the FDA, via electronic submission, on January 30, 2003, and confirmation of receipt was received on January 31, 2003 (NDA 021-476);

(c) Sepracor received an Approval Letter from the FDA for LUNESTA™ (eszopiclone) NDA 021-476 on December 15, 2004.

(11) "A brief description beginning on a new page of the significant activities undertaken by the marketing applicant during the applicable regulatory review period with respect to the approved product and the significant dates applicable to such activities;" 37 C.F.R. § 1.740(a)(11).

Attached is a chronology that briefly describes the significant regulatory activities and relevant dates associated with Sepracor Inc.'s efforts to seek approval of this product before the FDA (Exhibit B).

(12) "A statement beginning on a new page that in the opinion of the applicant the patent is eligible for the extension and a statement as to the length of the extension claimed, including how the length of extension was determined;" 37 C.F.R.§ 1.740(a)(12).

Statement of Eligibility of the Patent for Extension

It is the opinion of the Applicant that U.S. Patent No. 6,444,673 is eligible for an extension. This opinion is based on the following information on U.S. Patent No. 6,444,673:

(a) 35 U.S.C. § 156(a): U.S. Patent No. 6,444,673 claims the approved human drug product LUNESTA™ (eszopiclone).

(b) 35 U.S.C. § 156 (a)(1): The term of said patent has not expired prior to the submission of this application.

(c) 35 U.S.C. § 156 (a)(2): The term of said patent has never been previously extended under 35 U.S.C. § 156 (e)(l).

(d) This application for extension is in compliance with 37 C.F.R. § 1.740.

(e) 35 U.S.C. § 156(a)(4): The product, LUNESTA™ (eszopiclone), has been subject to a regulatory review period as defined in 35 U.S.C. § 156(g) before its commercial marketing or use.

(f) 35 U.S.C. § 156(a)(5)(A): The NDA for the product received approval under the provision of law (i.e., FDC Act §505) under which the applicable regulatory review occurred.

(g) This application was submitted within sixty (60) days from the December 15, 2004 NDA approval date.

(h) 35 U.S.C. § 156(c)(4): No other patent term has been extended for the same regulatory review period for this product.

Statement as to Length of Extension Claimed

The term of U.S. Patent No. 6,444,673 should be extended by 760 days (i.e. 2 years 30 days), or until February 14, 2014 (as 2012 is a leap year). This term of extension was determined on the following bases.

First, the following calculation of the regulatory period is in accordance with 35 U.S.C. § 156 and 37 C.F.R. § 1.775. The length of this extension was determined as follows:

The effective date of the Investigational New Drug (IND) application is August 21, 1999, which was thirty (30) days after FDA receipt of the IND on July 22, 1999. The IND number is 58,647.

The new drug application (NDA) for LUNESTA™ was initially submitted via electronic filing to the FDA on January 30, 2003 and receipt was acknowledged by the FDA on January 31, 2003.

An approval letter for the NDA was issued by the FDA on December 15, 2004.

U.S. Patent No. 6,444,673 issued on September 3, 2002, and is entitled to a patent term of 20 years from the earliest filing date (January 16, 1992).

As set forth in 35 U.S.C. § 156(g)(1)(B), the regulatory review period for a new drug equals the sum of the following periods (i) and (ii):

(i) The time between the date an exemption under §505(i) of the FFDCA became effective (the effective date of the IND) and the date an application was initially submitted under §505 of the FFDCA (the date of the initial submission of the NDA).

An IND for the product was effective on August 21, 1999. The NDA for the product was submitted on January 30, 2003. Thus, for the purpose of this calculation, item (i) for the product equals the number of days from August 21, 1999, to January 30, 2003, or 1258 days.

(ii) The time between the date an application was initially submitted under §505(b) of the FFDCA (the date of the initial submission of the NDA) and the date the application was approved (the approval date of the NDA).

The NDA for the product was submitted on January 30, 2003. The NDA was approved on December 15, 2004. Thus, for the purpose of this calculation, item (ii) equals the number of days from January 30, 2003, to December 15, 2004, or 685 days.

In accordance with 35 U.S.C. § 156(c), the term of a patent eligible for extension shall be extended by the time equal to the regulatory review period for the approved product which occurred after the date the patent issued. U.S. Patent No. 6,444,673 issued on September 2, 2002. For the portion of the regulatory review period calculated pursuant to item (i) above, the period occurring after issuance of the patent equals the number of days from September 2, 2002 to January 30, 2003, or 150 days. The entire regulatory review period calculated for item (ii) above occurred after the issuance date of the patent.

Second, 35 U.S.C. § 156(c)(1)-(3) also set forth the following exceptions which may operate to reduce the length of the review period used to calculate patent term extension.

(1) Each period is reduced by any period during which the applicant did not act with due diligence.

There has been no lack of due diligence during the period of regulatory review.

Accordingly, no reduction in the review period is required by this provision.

(2) Each period includes only one-half of the number of days in phase (i).

One-half of the number of days in phase (i) equals one-half of 150 days, or 75 days. Adding this number of days to the number of days in phase (ii) (685 days) results in a review period of 760 days.

(3) If the period remaining in the patent term after the date of approval of the approved product when added to the regulatory review period as revised under paragraphs (1) and (2) above exceeds fourteen years, the period of extension shall be reduced so that the sum of both such periods does not exceed fourteen years.

On the date of approval of the NDA for the product, December 15, 2004, 9 years and 146 days remained in the term of U.S. Patent No. 6,444,673. Adding this period to the review period calculated above yields a period of less than fourteen years. This provision, therefore, does not affect the period of extension to which U.S. Patent No. 6,444,673 is entitled.

Third, 35 U.S.C. §156(g)(6) limits the period of patent term extension to a maximum of five years from the original expiration date of the patent. The original expiration date of U.S. Patent No. 6,444,673 is January 16, 2012. Accordingly, the maximum extension allowed by this provision would extend the term to January 16, 2017. Extension of the patent by the number of days calculated above would not extend the patent beyond this date. Accordingly, this provision does not operate to shorten the period of extension to which U.S. Patent No. 6,444,673 is entitled.

Thus, U.S. Patent No. 6,444,673 is entitled to an extension of 760 days (i.e. 2 years and 30 days), to February 14, 2014.

(13) "A statement that applicant acknowledges a duty to disclose to the Director of the United States Patent and Trademark Office and the Secretary of Health and Human Services or the Secretary of Agriculture any information which is material to the determination of entitlement to the extension sought;" 37 C.F.R. § 1.740(a)(13).

Applicant acknowledges a duty to disclose to the Director of the United States Patent and Trademark Office and the Secretary of Health and Human Services or the Secretary of Agriculture any information which is material to any determination of entitlement to the extension sought.

(14) "The prescribed fee for receiving and acting upon the application for extension (see §1.20(j));" 37 C.F.R.§ 1.740(a)(14).

Pursuant to 37 C.F.R. § 1.20(j), a check in the amount of $1,120.00 is enclosed with this application.

Should additional fees be necessary in connection with the filing of this paper, or if a petition for extension of time is required for timely acceptance of same, the Director is hereby authorized to charge Deposit Account No. 08-1935 for any such fees. Should a refund of fee paid be necessary, the Director is hereby authorized to credit any such amount to Deposit Account No. 08-1935.

(15) "The name, address and telephone number of the person to whom inquiries and correspondence relating to the application for patent term extension are to be directed;" 37 C.F.R.§ 1.740(a)(15).

Please direct all inquiries and correspondence relating to this application for term extension to:

Philip E. Hansen
HESLIN ROTHENBERG FARLEY & MESITI, P.C.
5 Columbia Circle
Albany, New York 12203-5160
Telephone: (518) 452-5600
Facsimile: (518) 452-5579

(16) "The application under this section must be accompanied by two additional copies of such application (for a total of three copies)." 37 C.F.R.§ 1.740(b).

This application for patent extension, including its attachments, is being submitted as one original and two duplicate copies thereof.

Respectfully submitted,

February 11, 2005
Date

Philip E. Hansen
Agent for Applicants
Reg. No. 32,700

Chronology of Significant Activities Regarding IND 58,647 and NDA 21-476 for LUNESTA™ (eszopiclone) Tablets

(July 22, 1999 to December 15, 2004)

Date	Type	Activity
7-22-1999	**IND**	**Original IND Submission (12 volumes)**
7-28-1999	IND	Response to FDA Request for additional desk copies
7-29-1999	IND	General Correspondence: FDA communicates assignment of IND number 58,647.
7-30-1999	IND	FDA Letter acknowledging receipt of Original IND Submission.
8-17-1999	IND	FDA Request for clarification regarding Pharm Tox section
8-19-1999	IND	Response to FDA Request for clarification of Pharm Tox section:Vols. 5 & 6 resubmitted with pages reordered
8-25-1999	IND	FDA Request for information: reformatting TK information to tabular form
8-25-1999	IND	Response to FDA Request: reformatted to tabular form information regarding cause of death for animals in TK studies
8-30-1999	IND	FDA Request for information: clarification of data in table
8-30-1999	IND	Response to FDA Request: Corrections to table
9-13-1999	IND	FDA Communication granting permission to proceed with proposed clinical studies
10-13-1999	IND	Response to FDA Request: copy of labeling for ZIMOVANE distributed in the UK
12-7-1999	IND	Response to FDA Request for Information dated September 13, 1999, and Protocol Amendments: Change in Protocol (Amendments 1 and 2 to Protocol 190-001) and revised Protocol 190-001; New Protocol 190-002; and New Investigators (Ruckle and Stoltz) for Protocol 190-002
12-15-1999	IND	General Correspondence: Final toxicology reports (28-day mouse, rat, and dog studies) expected submission date; up-coming submission of a formal meeting request and information package
1-7-2000	IND	Protocol Amendments: Protocol 190-012, Amendment 1, and Modification Notice 1; New Protocol 190-026; and New Investigator (M. Cohn) for Protocol 190-012
1-18-2000	IND	General Correspondence: Proposed meeting with FDA regarding development of eszopiclone and intent to provide a pre-meeting package
1-27-2000	IND	Information Amendment: Pharm/Tox (study summaries and final reports for 1 pharmacology study and 12 toxicology studies submitted in draft in original IND, and study summaries and final reports for 6 drug metabolism studies provided as new information) (9 volumes)
2-7-2000	IND	Information Amendment: CMC (information for comparator drug)
2-10-2000	IND	General Correspondence: Meeting request by Sepracor to discuss development of eszopiclone with proposed outline information package
2-28-2000	IND	FDA Communication setting date of pre-NDA meeting: March 20, 2000
3-2-2000	IND	Protocol Amendment: New Investigators (B.Corser, M.Scharf, and J.Schwartz) for Protocol 190-026
3-7-2000	IND	Pre-Meeting Information Package for March 20, 2000 (hand-delivered to FDA)

Chronology of Significant Activities Regarding IND 58,647 and NDA 21-476 for LUNESTA™ (eszopiclone) Tablets

(July 22, 1999 to December 15, 2004)

Date	Type	Activity
3-8-2000	IND	Teleconference with FDA regarding details of planned meeting to discuss clinical development and proposed NDA
3-15-2000	IND	FDA Request for an additional reviewer desk copy of Serial No. 003, Volume 6, and questions regarding Studies 516 and 505
3-15-2000	IND	Response to FDA Request: Provided additional reviewer desk copy of Serial No. 003, Volume 6
3-16-2000	IND	Protocol Amendments: New Protocol (Protocol 190-005), and New Investigator (R.Stoltz) for 190-005
3-20-2000	IND	General Correspondence: Provided list of attendees for Monday, March 20, 2000 meeting
3-20-2000	IND	Pre-NDA Meeting with the FDA
3-27-2000	IND	Teleconference with FDA: Discussion of FDA comments regarding Protocol 190-026
3-28-2000	IND	General Correspondence: Questions from Sepracor regarding Protocol 190-026
3-29-2000	IND	FDA Request for Information: Formally submit March 28, 2000 questions in hard copy with a 1571 Form
4-4-2000	IND	Response to FDA Request: Formal submission of March 28, 2000 questions
4-4-2000	IND	FDA Letter: Pharm/Tox review of the original IND submission completed
4-26-2000	IND	General Correspondence: Status inquiry review of submitted information
4-27-2000	IND	General Correspondence: Sepracor sent additional questions regarding Study 190-026
5-5-2000	IND	Protocol Amendment: New Investigator (eight new principal investigators) for Protocol 190-026
5-24-2000	IND	Sepracor Minutes of March 20, 2000 Meeting with FDA and Submission of Presentation Slides
5-30-2000	IND	Protocol Amendment: New Investigators (Black, Rosenberg, Vollmer, and Zammit) for Protocol 190-026
5-31-2000	IND	General Correspondence: Follow-up regarding Sepracor requests for comments for Protocol 190-026; timing for an End-of-Phase 2 meeting, Aug-Sept; FDA finalizing its minutes from March 20, 2000 meeting
6-6-2000	IND	General Correspondence: Sepracor status inquiry regarding FDA comments for Protocol 190-026, and scheduling of meeting with FDA.
6-20-2000	IND	General Correspondence: Status update from FDA re Sepracor's questions
6-23-2000	IND	FDA Minutes of March 20, 2000 meeting between FDA review team and Sepracor
6-30-2000	IND	Information Amendment: Chemistry, Manufacturing, and Controls (update for drug product and placebo/diluent; & additional comparator drug and placebo to the same)
7-7-2000	IND	Protocol Amendments: Changes in Protocol Objectives (Amendment 1 to Protocol 190-026); Administrative Change 1 to Protocol 190-005; and New Investigator (P.Leese) for Protocol 190-005

Chronology of Significant Activities Regarding IND 58,647 and NDA 21-476 for LUNESTA™ (eszopiclone) Tablets

(July 22, 1999 to December 15, 2004)

Date	Type	Activity
7-18-2000	IND	General Correspondence: FDA called regarding status of review and circulating letter, and requested Sepracor provide preferred dates for End of Phase II meeting
7-18-2000	IND	General Correspondence: FDA scheduled End of Phase II meeting for September 21, 2000, and meeting package is due 3-4 weeks prior
7-19-2000	IND	Protocol Amendments: New Protocols; New Investigators for 190-010 (Abdou) and for 190-015 (Leese). Information Amendment: CMC alternate supplier of comparator and a new dosage form (film coated tablets)
8-18-2000	IND	Submission of draft Protocols 190-045 and 190-046 for FDA for Division review, advice, and comment. Protocol Amendment: New Protocol, New Investigator (N.Abdou) for Protocol 190-019
8-21-2000	IND	General Correspondence: informed FDA of August 18, 2000 submission and confirmed End of Phase II meeting for September 21, 2000
8-25-2000	IND	Submission of End of Phase II Pre-Meeting Information Package (1 volume)
8-28-2000	IND	General Correspondence: FDA confirmed receipt of End of Phase II Pre-Meeting Information Package.
9-1-2000	IND	Protocol Amendment: New Protocol (Protocol 190-045, Amendment 1); and New Investigator (B.Corser) for Protocol 190-045
9-5-2000	IND	General Correspondence: change in lab name for Protocols 190-010, 190-015, and 190-019
9-11-2000	IND	General Correspondence: FDA communicated that CMC portion of End of Phase II will be independent of and immediately following clinical/tox discussion
9-13-2000	IND	General Correspondence: FDA scheduled CMC portion of End of Phase II Meeting for September 26, 2000
9-15-2000	IND	General Correspondence: Discussion with FDA confirming issues to be discussed at the End of Phase II Meetings on September 21, 2000 (tox/clin/stats) and September 26, 2000 (CMC)
9-15-2000	IND	Protocol Amendment: New Protocols, New Investigators for Protocol 190-018 (T.Stock) and for Protocol 190-020 (P.Leese)
9-20-2000	IND	General Correspondence: FDA provides list of participants for End of Phase II meeting on September 21, 2000
9-20-2000	IND	General Correspondence: Sepracor provides Agenda, Sepracor attendees, and questions for FDA discussion for End of Phase II meeting on September 21, 2000
9-21-2000	IND	End of Phase II Meeting (tox/clin/stats) with FDA
9-22-2000	IND	FDA Request for Information: two desk copies of Protocol Amendments (Protocols 190-018 and 190-020)
9-26-2000	IND	End of Phase II Meeting (CMC) with FDA
9-27-2000	IND	Response to FDA Request: submission of two additional reviewer desk copies of Protocol Amendments (Protocols 190-018 and 190-020)
10-03-2000	IND	Protocol Amendment: New Protocol, New Investigator for Protocol 190-021 (N.Abdou)

Chronology of Significant Activities Regarding IND 58,647 and NDA 21-476 for LUNESTA™ (eszopiclone) Tablets

(July 22, 1999 to December 15, 2004)

Date	Type	Activity
10-5-2000	IND	FDA Request for Information: Sepracor's minutes of End of Phase II Meetings on September 21 and 26, 2000.
10-10-2000	IND	FDA Request for Information: provide details of abuse liability assessment evaluation current procedure
10-13-2000	IND	FDA Request for Information: desk copy of Protocol Amendment submitted October 3, 2000
10-13-2000	IND	Response to FDA Request: submitted desk copy of October 3, 2000 Protocol Amendment
10-18-2000	IND	FDA communication regarding review of September 15, 2000 submission
10-19-2000	IND	Protocol Amendment: New Protocols, New Investigators. Protocols 190-014, 190-022, 190-023, and 190-046; Investigator information for these 4 protocols; and 2 new Investigators for Protocol 190-045
10-24-2000	IND	FDA Letter regarding notification of content and file date requirements for annual report of progress
10-26-2000	IND	FDA Request for Information: desk copy of PK Protocols 190-022 and 190-023, and all future PK protocols
10-26-2000	IND	Response to FDA Request: submitted desk copy of PK Protocols 190-022 and 190-023
10-27-2000	IND	FDA Request for Information: another desk copy of October 3, 2000 Protocol Amendment
10-27-2000	IND	Protocol Amendment: New Protocol, New Investigator for Protocol 190-013 (G.Bloomgren)
10-30-2000	IND	Response to FDA Request: desk copy of October 3, 2000 Protocol Amendment
11-29-2000	IND	General Correspondence: list of FDA attendees at End of Phase II meetings
12-1-2000	IND	Response to FDA Request: submitted Sepracor's End of Phase II Meetings Minutes and Statistical Rationale for Protocol 190-046
12-5-2000	IND	FDA Request for Information: electronic copies of Sepracor's End of Phase II Meetings Minutes submitted on December 1, 2000
12-5-2000	IND	Response to FDA Request: provided electronic files containing the End of Phase II Meetings Minutes submitted on December 1, 2000
12-7-2000	IND	Submitted Annual Report covering the period of August 25, 1999, through August 31, 2000 (1 volume)
12-8-2000	IND	Protocol Amendment: New Protocol for Protocol 190-049
12-22-2000	IND	Information Amendment: Pharm/Tox (study summaries and final reports for two pharm studies, nine tox studies, and five drug metabolism studies) (22 volumes)
1-3-2001	IND	Protocol Amendment: Change in Protocol (Amendment 1 to Protocol 190-010); New Investigator (J.Walsh) for Protocol 190-045; and New Investigators (10) for Protocol 190-046
1-9-2001	IND	FDA Request for Information: desk copy of January 3, 2001 Protocol Amendment
1-9-2001	IND	Response to FDA Request: provided desk copy of January 3, 2001 Protocol Amendment

Chronology of Significant Activities Regarding IND 58,647 and NDA 21-476 for LUNESTA™ (eszopiclone) Tablets

(July 22, 1999 to December 15, 2004)

Date	Type	Activity
1-18-2001	IND	General Correspondence: Teleconference with initiated by FDA regarding status request for study 190-010 by Biopharmaceutics reviewer, & informing FDA study is complete
1-19-2001	IND	General Correspondence: Teleconference with initiated by FDA regarding status request for study 190-022 by Biopharmaceutics reviewer, & informing FDA study is complete. Also informed FDA that studies 190-011 and 190-048 will be submitted next week
1-25-2001	IND	General Correspondence: Sepracor status inquiry regarding End of Phase II Meetings Minutes and 190-046 statistical rationale, to which FDA indicated meeting minutes are within 2 weeks of finalization
1-25-2001	IND	FDA Communication providing comments and recommendations from the Biopharmaceutical reviewers regarding submissions of October 3, 19, & 27, 2000
2-22-2001	IND	FDA Final Minutes of End-of-Phase II meetings held September 21, 2000 (tox/clin/stats) and September 26, 2000 (CMC)
2-23-2001	IND	Information Amendment: CMC (additional film-coated tablet formulation)
3-1-2001	IND	General Correspondence: provided proposed format for presentation of Abuse Liability information section of NDA
3-9-2001	IND	General Correspondence: Notified FDA of up-coming two-volume submission that will include a request for review and comment
3-21-2001	IND	Sepracor requested FDA comments and advice on an IND and a request for a teleconference; Protocol Amendments: New Protocols (190-016 and 190-048); Change in Protocols (190-045, -046 and -049) and New Investigators for 190-046, -049 and –014
3-23-2001	IND	General Correspondence: Message from FDA regarding a new FDA medical reviewer and requesting recommendations for getting new reviewer up to speed
3-28-2001	IND	General Correspondence: Teleconference with FDA's new medical reviewer discussing general program
3-28-2001	IND	Response to FDA Request: send final FDA meeting minutes for the End of Phase II meetings held on September 21 and 26, 2000.
3-30-2001	IND	Protocol Amendment: New Protocol (Protocol 190-011);New Investigator (D.Morrison) for 190-011
4-9-2001	IND	Protocol Amendments: New Protocols (190-024 and 190-025); Revised Protocol (Amendment 1 and Revised Protocol for 190-049); New Investigators for 190-013, 190-024, 190-025, 190-046, and 190-049 (3 volumes)
4-12-2001	IND	Safety Reports: Event Nos. SU190049-010328.1.I and SU190049-010404.1.I
4-12-2001	IND	Information Amendment: CMC (3.0 mg film-coated tablet formulation and matching placebo; and over encapsulated active comparator drug (diazepam tablets) and matching placebo)
4-12-2001	IND	General Correspondence: FDA requesting results of the mutagenicity battery conducted with eszopiclone, and Sepracor responded that these assays were submitted in December 2000
4-19-2001	IND	General Correspondence: Teleconference with FDA Medical Reviewer for eszopiclone regarding teleconference scheduled for April 23, 2001

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(July 22, 1999 to December 15, 2004)

Date	Type	Activity
4-24-2001	IND	Sepracor submitted Briefing documents for teleconference scheduled for April 25, 2001
4-25-2001	IND	Sepracor provided 1 replacement page and one additional page for briefing documents for teleconference scheduled for April 25, 2001
5-4-2001	IND	Information Amendment: Pharmacology/Toxicology: Sepracor Document Nos. 190-831 and 190-832.
5-9-2001	IND	General Correspondence: Sepracor status inquiry to FDA regarding statistical rationales submitted for Protocols 190-045 and 190-046
5-10-2001	IND	Telephone Report for the Status of 190-045 Review.
5-14-2001	IND	General Correspondence: FDA indicated their review of statistical rationales is nearly complete
5-14-2001	IND	Protocol Amendment: New Investigators (initial investigators for Protocols 190-016 and 190-048; new investigators for Protocols 190-011 and 190-946)
5-14-2001	IND	FDA Minute for April 25, 2001 teleconference discussing current status of carcinogenicity assessment for eszopiclone
5-31-2001	IND	FDA Request for Information: clarification of Sepracor Document No. 190-818-2000
6-6-2001	IND	Protocol Amendment: New Protocols for Protocols 190-024, -025, -046, -048, and -049
6-18-2001	IND	Response to FDA Request: information requested by FDA May 31, 2001
6-22-2001	IND	Response to FDA Request: Information Amendment (Pharmacology/ Toxicology)
6-25-2001	IND	General Correspondence: faxed copy of cover letter of Response to FDA Request submitted on June 22, 2001
6-26-2001	IND	Fax for Submission of Carcinogenicity Rationale: Telephone conversations were held on June 20, 22, 25 and 26, 2001
6-27-2001	IND	FDA Letter regarding statistical review and providing comments regarding Protocols 190-045 and –046
7-3-2001	IND	IND Safety Reports: Initial Reports for Event Nos. SUI90049-010418-1, SUI90049-010516-1, SUI90049-010529-1, SUI90049-010420-1, SUI90049-010510-1, and SUI90049-010514-1
7-3-2001	IND	Response to FDA Request: answers to FDA questions regarding Studies 190-045 and –046
7-5-2001	IND	General Correspondence: Sepracor status inquiry regarding FDA response to Sepracor's July 3, 2001 submission regarding Study 190-045
7-6-2001	IND	IND Safety Report: Initial Report for Event No. SUI90049-010622-1
7-6-2001	IND	FDA Response to Sepracor's July 3, 2001 submission regarding Protocol 190-046
7-10-2001	IND	Response to FDA Request: response to FDA's June 27, 2001 letter regarding Protocols 190-045 and –046
7-10-2001	IND	General Correspondence: Correction to July 3, 2001 submission
7-18-2001	IND	Response to FDA Request: information concerning Protocols 190-045 and –046; submitted for discussion during July 18, 2001 FDA teleconference
7-24-2001	IND	Information Amendment: New Investigators (6) for 190-048
7-31-2001	IND	IND Safety Report: Initial Report for Event No. SUI90049-010718-1
7-31-2001	IND	IND Safety Report: Initial Report for Event No. SUI90048-010723-1
7-31-2001	IND	FDA Request for Information: requesting clarification of information for investigator
8-1-2001	IND	IND Safety Report: Initial Report for Event No. SUI90049-010715-1
8-2-2001	IND	Response to FDA Request for Information: provided clarification of information for investigator
8-2-2001	IND	Response to FDA Request :Information Amendment: Pharmacology / Toxicology
8-7-2001	IND	IND Safety Report: Initial Report for Event No. SU190049-010616-1
8-7-2001	IND	IND Safety Report: Follow-up Report for Event No. SU190049-010529-1
8-10-2001	IND	Response to FDA Request: Overall Clinical Development Program, Submission of Draft Clinical Protocol for Review and Comment

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(July 22, 1999 to December 15, 2004)

Date	Type	Activity
8-15-2001	IND	IND Safety Report: Initial Report for Event No. SU190048-010806-1
8-15-2001	IND	IND Safety Reports: Initial Report for Event No. SU190049-010726-1, and Follow-up Reports for Event Nos. SU190049-010514-1 and SU1900449-010715-1
8-21-2001	IND	IND Safety Reports: Initial Reports for Event Nos. SUI90049-0107222-1, SUI90049-010809-1, and SUI90049-010516-1
8-21-2001	IND	General Correspondence: Sepracor Request for Pre-NDA Meeting
8-23-2001	IND	General Correspondence: FDA Response to request for a Pre-NDA Meeting
8-23-2001	IND	General Correspondence: Sepracor's response to FDA's proposed date for Pre-NDA Meeting
8-24-2001	IND	IND Safety Report: Initial Report (Subject C-T/S002/R0313)
8-27-2001	IND	General Correspondence: Sepracor Request for separate CMC/Biopharm Pre-NDA Meeting
8-28-2001	IND	General Correspondence: Follow-up discussion regarding meeting request
9-4-2001	IND	General Correspondence: Scheduling requested CMC Pre-NDA Meeting
9-4-2001	IND	General Correspondence: Request for re-schedule CMC Pre-NDA Meeting due to religious holiday
9-5-2001	IND	General Correspondence: Rescheduled CMC Pre-NDA Meeting from September 27 to September 28, 2001
9-10-2001	IND	General Correspondence: Teleconference confirming CMC Pre-NDA Meeting details and number of Briefing Package copies needed
9-10-2001	IND	Submitted CMC Briefing Package for pre-NDA Type B Meeting
9-14-2001	IND	Response to FDA Request: Transmitted copy of Sepracor Minutes of March 20, 2000 Meeting with FDA and Submission of Presentation Slides originally sent May 24, 2000
9-17-2001	IND	IND Safety Reports: Follow-up Report (subject AAT/S003/R0099) and Follow-up Report (subject PGC/S013/R0672)
9-19-2001	IND	General Correspondence: Advise FDA of new Sepracor official correspondent for the eszopiclone IND, and Status Inquiry regarding two reviews currently underway by FDA
9-20-2001	IND	IND Safety Reports: Initial Reports (Subjects, MAD/S030/R0626 & MAM/S007/R0035)
9-20-2001	IND	Request for comments regarding the proposal for thyroid and estradiol levels in protocol 190-049
9-20-2001	IND	General Correspondence: Change of CMC Pre-NDA meeting venue from face-to-face to a teleconference
9-24-2001	IND	General Correspondence: Fax confirmation of teleconference details for CMC Pre-NDA meeting on September 28, 2001
9-25-2001	IND	General Correspondence: Requesting update on CMC Pre-NDA meeting and follow-up
9-26-2001	IND	General Correspondence: Update on CMC Pre-NDA Meeting participants and comments on carcinogenicity (CAC Meeting) and clinical study 190-047
9-28-2001	IND	General Correspondence: Facsimile to FDA regarding 2-Year mouse carcinogenicity study
9-28-2001	IND	CMC Pre-NDA Meeting with FDA (Teleconference)
10-01-2001	IND	General Correspondence: Teleconference regarding FDA's receipt of requested information from 2-Year mouse carcinogenicity study
10-3-2001	IND	Submission of Briefing Package for Pre-NDA Type B Meeting
10-9-2001	IND	General Correspondence: Submission of Sepracor's Pre-NDA CMC Meeting Minutes (Teleconference)
10-11-2001	IND	Protocol Amendments: Change in Protocols for 190-019, -018, and –020
10-12-2001	IND	IND Safety Report: Follow-up/Final Report (Subject ALL/S004/R0285)
10-12-2001	IND	IND Safety Reports: Follow-up Report (Subject C-T/S002/R0313), Amended Final Report (Subject KAH/S007/R0242), Follow-up Report (Subject SBK/S001/R0378), Initial Report (Subject KHM/S011/R0494), Initial & Follow-up Reports (Subject RPA/S033/R0616)

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(July 22, 1999 to December 15, 2004)

Date	Type	Activity
10-18-2001	IND	General Correspondence: Sepracor request for information from FDA on Pre-NDA Meeting
10-23-2001	IND	Submission of Revised and Additional Questions for Pre-NDA Meeting
10-23-2001	IND	General Correspondence: Teleconference confirming FDA's receipt of Updated Questions for Pre-NDA Meeting
10-25-2001	IND	General Correspondence: Sepracor request for information regarding FDA's Pre-NDA Review Team Meeting
10-25-2001	IND	Fax of Minutes of the Executive CAC meeting.
10-26-2001	IND	Protocol Amendments: New Protocol, Change in Protocol, New Investigators (6) for 190-047
10-29-2001	IND	FDA Pre-NDA Type B Meeting
10-31-2001	IND	General Correspondence: Submitted FDA Pre-NDA Meeting Minutes from October 29, 2001
11-1-2001	IND	General Correspondence: Pre-NDA Meeting Minutes Fax
11-6-2001	IND	FDA Internal Meeting Regarding Pre-NDA Package – FDA Request for follow-up teleconference on November 27, 2001
11-7-2001	IND	IND Safety Reports: Initial Report subject DJS/S002/R0282, Final Follow-up Report subject FWR/S004/R0012, and Follow-up Report subject VLW/S008/R0666
11-14-2001	IND	General Correspondence: Sepracor request for input on eSub/eNDA
11-16-2001	IND	FDA Letter with comments referencing Sepracor's August 10, 2001 request for comments
11-19-2001	IND	FDA Letter with comments referencing Sepracor's March 21, 2001 request for comments
11-20-2001	IND	Fax from the FDA providing copies of FDA Letters issued November 16 & 19, 2001
11-20-2001	IND	General Correspondence: Informed the Division of upcoming teleconference with Dr. Levin (FDA-CDER electronic submissions)
11-21-2001	IND	IND Safety Reports: Final Follow-up Report (Subject SBK/S001/R0378), Initial Report (Subject LTJ/S009/R0471), and Initial Report (TID/S005/R0285)
11-27-2001	IND	Pre-NDA Meeting Follow-up Teleconference with FDA
11-28-2001	IND	Response to FDA Request: Information to support monitoring of Estradiol levels in clinical studies.
11-28-2001	IND	Protocol Amendment: New Investigators for 190-047 (28), and Information Amendment: Pharmacology/Toxicology (5 reports)
11-29-2001	IND	Response to FDA Request: copy of November 28, 2001 Response to FDA Request
11-30-2001	IND	General Correspondence: Teleconference with FDA regarding follow-up on Pre-NDA Discussions and Re-Submission of the Estradiol Monitoring Rationale Information
11-30-2001	IND	Response to FDA Request: Information Amendment: Pharmacology/Toxicology (new animal study to evaluate hormonal levels)
12-3-2001	IND	General Correspondence: Submitted Sepracor's Electronic Submission (eSub)/Electronic NDA (eNDA) Meeting Minutes (Teleconference)
12-6-2001	IND	FDA Request for Information: Pre-NDA Meeting Teleconference
12-6-2001	IND	Information Amendment: Chemistry, Manufacturing, and Controls
12-7-2001	IND	General Correspondence: provided list of attendees at the November 27, 2001 Pre-NDA Follow-Up Meeting teleconference
12-7-2001	IND	Response to FDA Request: submitted Sepracor's Pre-NDA Follow-up Meeting Minutes (Teleconference)
12-7-2001	IND	Protocol Amendment: New Investigator (Pellegrino) for 190-047
12-10-2001	IND	General Correspondence: Providing list of outstanding pre-NDA questions
12-10-2001	IND	General Correspondence: discussing new Sepracor contact person for eszopiclone
12-17-2001	IND	General Correspondence: Sepracor Request for information on Preclinical Study Design

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(July 22, 1999 to December 15, 2004)

Date	Type	Activity
12-18-2001	IND	General Correspondence: Discussed outstanding questions with the FDA on Pre-NDA Meeting Package Questions
12-21-2001	IND	IND Safety Report: Initial Report (1)
1-3-2002	IND	General Correspondence: FDA notice pre-assigning NDA Number 21-476 to Eszopiclone NDA
1-4-2002	IND	Protocol Amendments: Change in Protocol, Revised Protocol 190-047
1-4-2002	IND	General Correspondence: Multiple teleconferences with FDA regarding Preclinical Study Design
1-8-2002	IND	General Correspondence: FDA responses to Preclinical Study Design & Pre-NDA Minutes
1-8-2002	IND	FDA Letter regarding FDA comments after review of IND for eszopiclone, and comments on the also on the November 30, 2001 Information Amendment
1-8-2002	IND	FDA Letter providing FDA's Minutes from October 29, 2001 Pre-NDA Meeting
1-9-2002	IND	General Correspondence: Follow-up with FDA on FDA's responses to Preclinical Study Design and Pre-NDA Minutes
1-24-2002	IND	General Correspondence: FDA stating that entire toxicology section must be duplicated in the NDA
2-21-2002	IND	IND Safety Report: Follow-Up Report (Subject LTJ/S009/R0471) for Study 190-049
2-26-2002	IND	General Correspondence: Discussion of 3 mg tablets
2-26-2002	IND	Protocol Amendments: Change in Protocol for 190-049 (Amendment 3), New Investigators (190-049-3, 190-013-2, 190-014-1, 190-026-1, 190-045-3, 190-046-3, 190-047-15, and 190-048-6)
2-27-2002	IND	IND Safety Report: Initial Report (JHD/S018/R0599) for Study 190-049
2-28-2002	IND	IND Safety Report: Initial Report (Subject S002/R0410/M-B) for Study 091-049
3-1-2002	IND	General Correspondence: Discussion of CMC stability data formatting, paper review copies for NDA, and new FDA Chemistry Team Leader
3-1-2002	IND	IND Safety Report: Initial Report (Subject D717/R0233/AAB) for Study 190-047
3-3-2002	IND	FDA Letter providing comments on Protocol Amendments submitted on October 26, 2001
3-4-2002	IND	General Correspondence: Follow-up discussion with FDA regarding CMC Review Aids and paper review copies
3-6-2002	IND	IND Safety Report: Initial Report (Subject DJB/S710/R0404) and Initial Report (Subject MAE/S014/R0694) for Protocols 190-047 and 190-049
3-7-2002	IND	General Correspondence: Proposal for providing CMC NDA Review aids per March 4, 2002 teleconference with FDA
3-11-2002	IND	Official FDA Meeting Minutes from the CMC End of Phase II meeting held on September 28, 2001
3-14-2002	IND	General Correspondence: USAN name eszopiclone adopted in place of previous chemical name (esopiclone)
3-15-2002	IND	IND Safety Report: Follow-up report (Subject TID/S005/R0285)
3-15-2002	IND	IND Annual Report for September 1,2000, through August 31, 2001
3-26-2002	IND	General Correspondence: FDA called to follow-up on the content of the proposal for CMC NDA Review Aids Sepracor provided on March 7, 2002
3-29-2002	IND	IND Safety Reports: Initial report for 190-047 (Subject WTB/S173/R0103), 2 Follow up reports for 190-049 (RDA/S033/R0616 & DRH/S025/R0636)
3-29-2002	IND	Response to FDA Request: Information relating to Protocols 190-016 and -047
4-4-2002	IND	IND Safety Reports: 2 Follow up reports for 190-047 (Subjects AAB/S717/R0233 & DJB/S710/R0494). Two initial and one follow up reports for 190-049 (Subjects ETL/S011/R0008, TPH/S007/R0665, & MAE/S014/R0694)

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(July 22, 1999 to December 15, 2004)

Date	Type	Activity
4-10-2002	IND	IND Safety Reports: Follow up report for 190-047 (Subject DJB/S710/R0494), three follow up reports for (Subjects TPH/S007/R0494, MAM/S007/R0035, and WTB/S714/R0103)
4-18-2002	IND	General Correspondence: Request for Type A Meeting to discuss plans to submit NDA with nonclinical data on mechanistic studies of hormonal changes per FDA's November 27, 2001 request
4-22-2002	IND	IND Safety Reports: Initial Report (Subject MTG/S714), Initial Report (Subject CAK/S022/R0660), Follow-Up Report (Subject CAK/S022/R0660) for Studies 190-047 and 190-049
4-25-2002	IND	General Correspondence: Request FDA's Endocrinology Review of Clinical Protocols
4-26-2002	IND	Protocol Amendment: New Investigators (190-046) and Other Investigators – Revised Forms FDA 1572 (190-024, -046, -047, & -049)
5-6-2002	IND	IND Safety Reports: Follow-up Report (Subject MTG/S714), Follow-up Report (Subject JHD/S018/R0599) for Studies 190-047 and 190-049
5-6-2002	IND	Protocol Amendments: Change in Protocol, Revised Protocol 190-049
5-9-2002	IND	IND Safety Reports: Initial Report (Subject GAR/S008/R0222), Initial Report (Subject L-H/S007/R0486) for Study 190-049
5-9-2002	IND	Information Amendment: Pharmacology/Toxicology Request to Attend the Carcinogenicity Peer Review meeting
5-10-2002	IND	General Correspondence: FDA acknowledged Sepracor's invitation to FDA to attend a Peer Review meeting
5-20-2002	IND	IND Safety Reports: Follow-up Report (Subject MTG/S714), and Follow-up Report (Subject ETL/S011/R0008) for Studies 190-047 and 190-049
5-21-2002	IND	General Correspondence: Teleconference with FDA regarding (1) report of Peer Review meeting; (2) FDA response on assessment of the design and collection of human hormone data; and (3) Sepracor's change of address
5-22-2002	IND	FDA Letter providing comments regarding Protocols 190-046, -048, and -049
5-30-2002	IND	Notification of change in sponsor's address and relocation downtime of phone and electronic systems
6-14-2002	IND	IND Safety Reports: Follow-up Report (Subject L-H/S007/R0486), and Final Follow-up Report (Subject S011/R0008/ETL)
6-24-2002	IND	IND Safety Reports: Initial Report (Subject S018/R0732/JLH), and Follow-up Report (Subject GAR/S007/R0222) for Study 190-049
7-10-2002	IND	General Correspondence: Teleconference with FDA regarding FDA's new Chemistry Team Leader and request for additional desk copy of CMC section of NDA
7-19-2002	IND	Protocol Amendment: New Investigator (190-049), IND Sponsor Responsibilities: Termination of Investigator Participation (B.Lewis)
7-23-2002	IND	IND Safety Report: Follow-up Report (Subject JLH/S018/R0732)
8-7-2002	IND	General Correspondence: Request for Type B Meeting to Discuss and Seek the Division's Input on Pharmacology/Toxicology Issues
8-8-2002	IND	General Correspondence: Teleconference with FDA confirming receipt of Type B Meeting Request Letter, and discussing possible meeting dates
8-8-2002	IND	IND Safety Report: Follow-up Report (Subject GAR/S007/R0222)

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(July 22, 1999 to December 15, 2004)

Date	Type	Activity
8-20-2002	IND	General Correspondence: Follow-up on Type B Meeting Request proposed dates for the end of September/early October 2002
8-21-2002	IND	General Correspondence: FDA contacted Sepracor regarding its decision to deny Sepracor's request for a Type B Meeting at this time
8-26-2002	IND	General Correspondence: Teleconference with FDA regarding Request for Information relating to Pharmacology/Toxicology and clarification of the Type B Meeting request.
8-26-2002	IND	FDA Letter providing comments concerning Protocol 190-016
8-29-2002	IND	FDA Letter of confirmation of FDA denial to Sepracor's request for a Type B Meeting
9-4-2002	IND	FDA Request for Information: Requested full reports of studies 190-870, Pathology Working Group, and $p53^{+/-}$ knockout mouse studies
9-5-2002	IND	Response to FDA Request: Provided by facsimile information requested by FDA on September 4, 2002
9-5-2002	IND	General Correspondence: Teleconference with FDA confirming FDA's receipt of information, and timing for submission of additional information
10-3-2002	IND	Response to FDA Request: Submission of Preclinical Package with requested information on Pharmacology/Toxicology and Type B Meeting Request (11 volumes)
10-10-2002	IND	General Correspondence: Teleconference with FDA confirming receipt of Sepracor's October 3, 2002 Preclinical Package
10-23-2002	IND	General Correspondence: FDA contacted Sepracor regarding logistics for eszopiclone NDA Meeting in December 2002, and guidelines for the length of briefing package and length of the meeting
10-24-2002	IND	General Correspondence: Fax to FDA memorializing October 23, 2002 teleconference discussion regarding the upcoming NDA Meeting
11-7-2002	IND	General Correspondence: FDA's list of FDA Attendees for December 17, 2002 meeting
11-20-2002	IND	General Correspondence: Teleconference with FDA on the meeting logistics and information package for December 17, 2002 meeting
11-22-2002	IND	Submission of Information Package for TYPE B Meeting to discuss issues relating to eszopiclone NDA Submission
11-25-2002	IND	Submitted fax revisions November 22, 2002 Information Package
12-3-2002	IND	General Correspondence: Request for specific attendee at NDA Meeting on December 17, 2002
12-6-2002	IND	General Correspondence: Teleconference to discuss request for particular attendee NDA Meeting on December 17, 2002
12-10-2002	IND	General Correspondence: Follow Up on the Request for particular attend and provision of additional information
12-12-2002	IND	Addendum to TYPE B Meeting Information Package: Pertinent review articles and presentation slides
12-13-2002	IND	Addendum II to TYPE B Meeting Information Package: Follow up to Addendum to the Meeting Information Package for December 17, 2002 Meeting
12-16-2002	IND	General Correspondence: Follow up regarding Addendum II to TYPE B Meeting Information Package

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(July 22, 1999 to December 15, 2004)

Date	Type	Activity
12-17-2002	IND	Type B NDA Meeting with the FDA
12-23-2002	IND	General Correspondence: Queries regarding outstanding issues for eszopiclone NDA submission
1-8-20003	IND	General Correspondence: Teleconference with FDA regarding December 23, 2002 discussion of queries regarding outstanding issues for eszopiclone NDA
1-10-2003	IND	General Correspondence: Teleconference with FDA regarding provision of Sepracor's Meeting Minutes from the December 17, 2002 meeting
1-10-2003	IND	Submission of Sepracor's Meeting Minutes from December 17, 2002 Eszopiclone NDA meeting
1-10-2003	IND	General Correspondence: Teleconference with FDA regarding outstanding issues concerning the eszopiclone NDA
1-13-2003	IND	General Correspondence: FDA contacted Sepracor to discuss outstanding questions concerning the eszopiclone NDA
1-15-2003	IND	FIELD COPY REQUIREMENTS – New England District Office Requirements for Eszopiclone NDA Field Copy
1-16-2003	IND	Sepracor's Payment of User Fee (fee for original new drug application requiring clinical data)
1-17-2003	IND	General Correspondence: Notice to provide paper copies and/or CD-Rom copies in addition to the full electronic NDA (NDA 21-476)
1-30-2003	**NDA**	**Original NDA (eNDA) Submission**
1-30-2003	NDA	Original NDA Submission (Field Copy)
1-31-2003	NDA	General Correspondence: Teleconference with FDA confirming Sepracor's submission of eNDA on January 30, 2003
2-5-2003	NDA	General Correspondence: Multiple teleconferences with FDA to follow-up with FDA after the receipt of the eszopiclone NDA
2-10-2003	NDA	General Correspondence: Teleconference with FDA to answer questions concerning timing of NDA/IND Annual Reports
2-12-2003	IND	Official FDA Meeting Minutes from December 17, 2002 Eszopiclone Pre-NDA Meeting
2-12-2003	NDA	General Correspondence: FDA contacted Sepracor regarding eszopiclone's status as a new chemical entity (NCE)
2-27-2003	NDA	General Correspondence: FDA contacted Sepracor to advise that a new medical reviewer was assigned to the eszopiclone NDA
2-27-2003	NDA	General Correspondence: FDA contacted Sepracor to request Sepracor send the new medical reviewer historical information between FDA and Sepracor
3-6-2003	NDA	General Correspondence: Teleconference with FDA regarding FDA's response to the two proposed submissions for the NDA
3-10-2003	NDA	General Correspondence: Teleconference with FDA regarding format for eszopiclone NDA Amendment
3-11-2003	NDA	Response to FDA Request: Information concerning Aventis IND 19,258 withdrawal
3-12-2003	NDA	FDA Request for Information: additional information concerning studies for the eszopiclone NDA
3-12-2003	NDA	Submission: Amendment 1 to NDA - Preclinical Minor Amendment NDA Section 5J – Corrected Datasets for Studies 190-833 and 190-834

Chronology of Significant Activities Regarding IND 58,647 and NDA 21-476 for LUNESTA™ (eszopiclone) Tablets

(July 22, 1999 to December 15, 2004)

Date	Type	Activity
3-13-2003	NDA	Response to FDA Request: Information requested concerning Eszopiclone NDA
3-13-2003	NDA	Response to FDA Request: Facsimile sending requested nonclinical information concerning Eszopiclone NDA
3-13-2003	NDA	General Correspondence: Communication with FDA regarding clarification of the subject for the study reports recently submitted
3-17-2003	NDA	Submission: Electronic CMC Review Aid
3-17-2003	NDA	General Correspondence: FDA contacted Sepracor to set up a Teleconference for March 18, 2003 to discuss issues relating to Eszopiclone NDA
3-18-2003	NDA	Teleconference with the FDA discussing issues pertaining to Eszopiclone NDA
3-18-2003	NDA	Submission: PDF File Containing a List of Clinical Study Report ERRATA
3-19-2003	NDA	General Correspondence: Communication with the FDA regarding expected timing for receipt of requested package of clinical related queries from March 18, 2003 Teleconference
3-19-2003	NDA	Submission: Response to March 18, 2003 Request for information
3-21-2003	NDA	Information for further clarification of clinlab and ECG collections
3-24-2003	NDA	General Correspondence: Teleconference with the FDA to discuss the Clinical Study Reports that have separate files of errata
3-24-2003	NDA	Clarification of the Clinical Study Reports submitted on March 19, 2003
3-24-2003	NDA	General Correspondence: Follow-up to Teleconference of March 24, 2003
3-24-2003	NDA	Submission: Response to Request for Information - Clinical Information
3-25-2003	NDA	Response to Request for Information: Clinical Information
3-25-2003	NDA	General Correspondence: Teleconference with the FDA to discuss the potential fileability issues identified by the FDA
3-25-2003	NDA	Submission: Amendment 2 to NDA: Errata Incorporated in Clinical Study Reports for Studies 190-012, 190-023, 190-026, 190-045 and 190-048 and Revised ISS Dataset
3-26-2003	NDA	General Correspondence: Confirmation FDA's receipt of March 25, 2003 submission
3-27-2003	NDA	FDA Request for Information: copy of the Cover letter and 356h form from the March 25, 2003 submission
3-27-2003	NDA	Response to FDA Request: Provided copy of the cover letter and 356h form for the March 25, 2003 submission
3-27-2003	NDA	General Correspondence: Teleconference with FDA to discuss the Filing Date, PDUFA Date and 74-Day Letter for NDA 21-476.
3-31-2003	NDA	General Correspondence: Confirm the site of Eszopiclone Drug Substance Release and the Drug Product Release
4-1-2003	NDA	General Correspondence: Teleconference with FDA confirm receipt of FDA queries on BioPharm NDA Section 6
4-3-2003	NDA	General Correspondence: FDA request for information to initiate Good Clinical Practices (GCP's) audits at three (3) clinical study sites
4-14-2003	NDA	FDA Filing Review Letter
4-15-2003	NDA	Submission: Request for Division Comments on Additional ISS Analyses, and Outline for Consolidated Literature Review
4-16-2003	NDA	General Correspondence: electronic copy of FDA Filing Review Letter for eszopiclone

Chronology of Significant Activities Regarding IND 58,647 and NDA 21-476 for LUNESTA™ (eszopiclone) Tablets

(July 22, 1999 to December 15, 2004)

Date	Type	Activity
4-18-2003	NDA	General Correspondence: FDA contacted Sepracor to clarify the FDA Biopharm Reviewers Request for Information
4-21-2003	NDA	Submission: Response to FDA Request - Information on three Clinical Study sites for Good Clinical Practices (GCPs) audits
4-22-2003	NDA	FDA BioPharm responses to the clarification on FDA queries
4-23-2003	NDA	General Correspondence: Clarification on the electronic submission of Eszopiclone NDA 21-476 format and content of 120-day Safety Update
4-28-2003	NDA	General Correspondence: Teleconference with FDA regarding follow-up on human endocrine study
5-2-2003	NDA	General Correspondence: FDA contacted Sepracor regarding reviewer for 120-day Safety Update eSUB Outline
5-2-2003	NDA	General Correspondence: FDA contacted Sepracor to follow-up on Pharm/Tox Study
5-5-2003	NDA	General Correspondence: Multiple communications concerning the teleconference on human endocrine study protocol
5-9-2003	NDA	General Correspondence: FDA-CDER Electronic Submission Specialist accepts Sepracor's NDA 21-476 120-day Safety Update Format Hierarchy
5-12-2003	NDA	General Correspondence: Sepracor request for a teleconference to discuss CMC issues and updates.
5-14-2003	NDA	General Correspondence: Communication with FDA to review attendees from both Sepracor and FDA for May 21, 2003 teleconference to discuss Protocols 190-041 and -042
5-15-2003	NDA	General Correspondence: Sepracor contacted FDA to follow-up on request for teleconference to discuss CMC issues and updates, and schedule the same
5-16-2003	NDA	General Correspondence: FDA contacted Sepracor to confirm the CMC issues and updates teleconference for May 19, 2003
5-19-2003	NDA	Teleconference between FDA and Sepracor regarding CMC issues and updates
5-19-2003	NDA	General Correspondence: FDA contacted Sepracor indicating that FDA's endocrine consultant will attend May 21, 2003 teleconference
5-21-2003	NDA	Teleconference between FDA and Sepracor regarding Protocols
5-21-2003	NDA	FDA Request For Information: complete address of vendor
5-23-2003	NDA	General Correspondence: Sepracor informed FDA the Full Response to the FDA BioPharm Review Team will be provided by June 2, 2003
5-28-2003	NDA	General Correspondence: FDA contacted Sepracor regarding timing for Sepracor's response to the 74-Day Letter
5-28-2003	NDA	Response to FDA Request: provided complete address for vendor
5-29-2003	NDA	Submission: Amendment to NDA - Clinical Amendment Submission of Amended Clinical Study Report 190-046
6-2-2003	NDA	General Correspondence: Sepracor contacted FDA regarding timing of the 120-Day Safety Update and responses to 74-Day Letter
6-5-2003	NDA	Submission: Sepracor's Meeting Minutes from May 19, 2003 CMC Teleconference
6-12-2003	NDA	General Correspondence: Sepracor contacted FDA to discuss response to BioPharm and Pharm/Tox queries by electronic submission
6-13-2003	NDA	Submission: Amendment to NDA - Response to Biopharmaceutical Queries

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(July 22, 1999 to December 15, 2004)

Date	Type	Activity
6-16-2003	NDA	General Correspondence: FDA contacted Sepracor with questions regarding location of study file
6-18-2003	NDA	Submission: Response to Pharmacology/Toxicology Reviewer Request on Historical Control Data
6-23-2003	NDA	General Correspondence: FDA contacted Sepracor to discuss FDA's questions regarding the recent electronic submission on validation reports
6-24-2003	NDA	Submission: Plan for Response to Filing Review Letter
6-24-2003	NDA	Teleconference with FDA BioPharm Reviewer to clarify outstanding issues relating to submission of validation reports
6-30-2003	NDA	Submission: Amendment to NDA - 120-Day Safety Update
6-30-2003	NDA	General Correspondence: FDA contacted Sepracor to discuss FDA BioPharm response to Sepracor's Follow-Up relating to submission of validation reports
7-3-2003	NDA	General Correspondence: Communications with FDA transmitting BioPharm information on validation reports
7-8-2003	NDA	FDA Request for Information: additional Biopharm questions
7-8-2003	NDA	Submission: Additional Response to Biopharmaceutical Queries
7-9-2003	NDA	General Correspondence: FDA contacted FDA to follow-up Division of Neuropharmacological Drug Product questions
7-10-2003	NDA	General Correspondence: FDA contacted Sepracor to follow-up on inquiries of data files the Original NDA 21-476
7-10-2003	NDA	Response to FDA Request: providing response to July 8, 2003 BioPharm request
7-10-2003	NDA	General Correspondence: Sepracor presented questions to new FDA Regulatory Project Manager
7-11-2003	NDA	General Correspondence: providing FDA with additional information concerning study
7-15-2003	NDA	Submission: Amendment to NDA - Chemistry, Manufacturing and Controls (stability updates)
7-15-2003	NDA	Submission: Amendment to NDA: Chemistry, Manufacturing and Controls (stability updates) – FIELD COPY
7-16-2003	NDA	General Correspondence: Sepracor contacted FDA to follow up on Sepracor's queries and changes at the Division
7-17-2003	NDA	General Correspondence: FDA contacted Sepracor concerning supporting evidence in the Original NDA 21-476 annotated label under special population
7-18-2003	NDA	General Correspondence: Follow-up on discussions regarding Annotated Label – Special Population of original NDA
7-21-2003	NDA	General Correspondence: communications relating to code FDA used for SAS meta analysis
7-22-2003	NDA	General Correspondence: Communication relating to clarification on the use of the code for SAS meta analysis.
7-23-2003	NDA	General Correspondence: provided location of code for SAS meta analysis
7-24-2003	NDA	FDA Request: clarification of questions regarding assessment of postmarketing cancer reports provided in 120-day Safety Update
7-25-2003	NDA	Submission: Electronic CMC Review Aid and Paper Copy of CMC Amendment dated July 15, 2003

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(July 22, 1999 to December 15, 2004)

Date	Type	Activity
7-29-2003	NDA	Response to FDA Request: Sepracor's plan of response to FDA's July 24, 2003 request, and submission of Sepracor questions regarding division changes
7-30-2003	NDA	General Correspondence: FDA correspondence referencing July 25, 2003 submission redirecting mailing of e-submissions
7-31-2003	NDA	General Correspondence: Request for teleconference from FDA Pharm/Tox Reviewer on upcoming nonclinical submissions
7-31-2003	NDA	FDA Response to July 29, 2003 questions regarding changes at the Neuropharm division and query on program/software used in data analysis.
8-4-2003	NDA	Response to FDA Request: provided information regarding study site audits
8-4-2003	NDA	General Correspondence: Questions for upcoming nonclinical submission teleconference.
8-6-2003	NDA	General Correspondence: teleconference scheduling with Pharm/Tox Reviewer on upcoming nonclinical submissions
8-6-2003	NDA	FDA Request for Information: pharm/tox sample collection times
8-7-2003	NDA	Response to FDA Request: provide clarification of sample collection times
8-7-2003	NDA	General Correspondence: scheduling of teleconference
8-7-2003	NDA	Response to informal observations made during Bioresearch Monitoring Inspection July 15-29, 2003
8-8-2003	NDA	General Correspondence: Clarification on use of SPSS software
8-14-2003	NDA	General Correspondence: confirmation of receipt of request for information
8-25-2003	NDA	General Correspondence: follow-up to requested re-analysis of bioequivalence study
8-27-2003	NDA	FDA Request for Information: clarification of USAN name for eszopiclone
8-27-2003	NDA	Response to FDA Request: provided USAN adoption letter for eszopiclone and print out of the official name listing
8-27-2003	NDA	FDA Request for Information: additional information on biostats, 120-day safety update, and pharm/tox teleconference issues
8-28-2003	NDA	Submission: Amendment to NDA: Pharmacology/Toxicology Amendment 6
8-28-2003	NDA	Submission: Amendment to NDA: Response to Filing Review Letter (74-Day Letter)
8-28-2003	NDA	General Correspondence: issues pertaining to the upcoming teleconference on September 3, 2003
8-28-2003	NDA	FDA Letter finding Sepracor to be compliant with applicable statutory requirements per FDA inspection (7/15-7/29/03)
8-28-2003	NDA	General Correspondence: Follow-up to confirming sufficiency bioequivalence analysis of 2 mg and 3 mg Clinical Service and Intended-for-Market Formulations
8-29-2003	NDA	Response to FDA Requests: regarding biostats, 120-day safety update, and pharm/tox issues
9-3-2003	NDA	Teleconference between FDA and Sepracor
9-4-2003	NDA	Response to questions regarding location of metabolic scheme for zopiclone
9-4-2003	NDA	Response to request minutes of eCAC meeting re: zopiclone
9-5-2003	NDA	Report encompassing communications from September 4-5, 2003
9-5-2003	NDA	Response to FDA Request: information requested during September 3, 2003 teleconference

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(July 22, 1999 to December 15, 2004)

Date	Type	Activity
9-5-2003	NDA	Response to FDA Request: Follow-up on request for information on metabolic scheme for eszopiclone and drug-drug interactions
9-10-2003	NDA	General Correspondence: provided requested guidance regarding locating raw data original NDA
9-11-2003	NDA	Response to FDA Request: provide response to September 3, 2003 teleconference questions
9-12-2003	NDA	Response to FDA Request: complete response to queries on post marketing cancer reports in 120 day Safety Update
9-12-2003	NDA	FDA Request for Information: questions on Pharm/tox CAC data
9-12-2003	NDA	Response to FDA Request: regarding pharm/tox CACA data
9-15-2003	NDA	Request for further clarification on SAS code provided September 5, 2003
9-16-2003	NDA	FDA reply and comment on information to be sent to FDA by September 18, 2003
9-17-2003	NDA	Response to FDA Request: BioPharm query
9-18-2003	NDA	FDA Request for Information: regarding SAS programs
9-19-2003	NDA	General Correspondence: Action plan regarding mouse and rat carcinogenicity studies
9-22-2003	NDA	Response to FDA Request: supplied SAS program code per September 18, 2003 request
9-24-2003	NDA	General Correspondence: Sepracor request for teleconference with clinical/statistical reviewers
9-24-2003	NDA	General Correspondence: follow-up with FDA on action plan to respond to FDA queries
9-24-2003	NDA	General Correspondence: FDA agrees to a face-to-face meeting to discuss the evidentiary basis for longer term administration claim for and to confirm that no Advisory Committee is necessary
9-25-2003	NDA	General Correspondence: Follow-up with FDA on Action Plan submitted on September 19, 2003
9-30-2003	NDA	General Correspondence: FDA response to Sepracor's Action Plan regarding mouse and rat carcinogenicity studies
9-30-2003	NDA	General Correspondence: Sepracor's response to FDA request for all inquires to go through program director
9-30-2003	NDA	General Correspondence: FDA advised it will be delayed in sending questions from pharm/tox reviewers
9-30-2003	NDA	FDA Request for Information: Questions from FDA Controlled Substance Staff on Statistics
10-1-2003	NDA	FDA Request for Information: Question from FDA Pharm/Tox Reviewer
10-1-2003	NDA	FDA response to Sepracor's September 19, 2003 communication referencing resubmission of carcinogenicity data files for NDA 21-476
10-1-2003	IND	General Correspondence: FDA advises that eszopiclone IND 58,647 Transferred from the Division of Neuropharmacological Drug Products to the Division of Anesthetic, Critical Care and Addiction Drug Products
10-1-2003	IND	General Correspondence: Follow-up with FDA on teleconference scheduling with the Division of Neuropharmacological Drug Products for Phase 3b protocols and accompanying questions
10-1-2003	NDA	Discipline Review Letter: Outlining 28 deficiencies in the CMC section of the NDA

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(July 22, 1999 to December 15, 2004)

Date	Type	Activity
10-2-2003	IND	Request for Type A Meeting to discuss matters relating to our pending application and new clinical trials
10-3-2003	IND	General Correspondence: Follow-up with FDA regarding October 2, 2003 request for Type A Meeting
10-6-2003	IND	FDA Notice that eszopiclone IND transfer is complete, and the Type A Meeting request is approved and will tentatively be held on January 14, 2004
10-7-2003	IND	FDA declines request for Type A Meeting (as requested October 2, 2003)
10-8-2003	IND	General Correspondence: Follow-up with of Neuropharm on the e-mail response of October 7, 2003 to a request for a Type A Meeting
10-10-2003	IND	General Correspondence: Meeting Request to the Division of Neuropharmacological Drug Products
10-10-2003	NDA	Response to FDA Request: regarding FDA's October 1, 2003 question concerning pharm/tox
10-14-2003	NDA	Submission: Request for Teleconference in Response to CMC Discipline Review Letter
10-14-2003	NDA	Nonclincal Response: Datasets of mouse and rat carcinogenicity studies
10-16-2003	NDA	Submission: Amendment to NDA: Biopharmaceutical Information Submission of Amended Clinical Study Report
10-16-2003	NDA	Response to FDA Request: information regarding data analysis
10-22-2003	NDA	FDA Letter - Response to request for meeting with DNDP
10-22-2003	NDA	FDA Request for Information: additional request from pharm/tox reviewer
10-23-2003	NDA	Response to FDA Request: provided response to October 22, 2003 pharm/tox request
10-23-2003	IND	FDA Letter - Confirming Type C Meeting (teleconference) between DACCADP, DNDP and Sepracor on January 14, 2004
10-24-2003	NDA	General Correspondence: provide a copy of a new journal article and a corresponding editorial article
10-24-2003	NDA	General Correspondence: Follow-up with FDA Division of Neuropharm
10-24-2003	IND	General Correspondence: request for a teleconference/written feedback from the Division of Anesthetic, Critical Care and Addiction Drug Products
10-29-2003	IND	General Correspondence: Response from FDA to requests from Sepracor for Division of Anesthetics feedback
10-31-2003	IND	General Correspondence: Clarification of Request to Division for input
11-10-2003	NDA	General Correspondence: Follow-up with FDA (Division of Neuropharm) on Sepracor's request for information – ECAC meeting, proprietary name and PDUFA action letter logistics
11-11-2003	NDA	Submission: Plan for Response to Chemistry Discipline Review Letter
11-13-2003	Press Release	Stating that the FDA has extended the review timetable for eszopiclone, changing it from November 29, 2003 to February 29, 2004
11-13-2003	NDA	General Correspondence: Follow-up with FDA on PDUFA Extension
11-14-2003	NDA	General Correspondence: Request to submit certain e-mail communications (with attachments) to NDA in accordance with electronic submission guidance.
11-19-2003	IND	Submission: Type C Meeting Confirmation
11-19-2003	NDA	General Correspondence: Request information on logistics of NDA transfer from the Division of Neuropharm to the Division of Anesthetic

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(July 22, 1999 to December 15, 2004)

Date	Type	Activity
11-24-2003	NDA	Submission: Nonclinical Studies Cross-Referenced to NDA 21-476
11-24-2003	NDA	General Correspondence: Proposed teleconference with FDA to Discuss CMC Issues on December 8, 2003
11-25-2003	NDA	Submission: Amendment to NDA Electronic (eNDA) Submission of Clinical and Pharmacology/Toxicology Data Currently under Review
11-26-2003	NDA	FDA Request for Information: Update on status of rat reproductive study
11-28-2003	NDA	FDA Letter: Review extension letter, extending from November 29, 2003 to February 29, 2004
12-3-2003	NDA	Response to FDA Request: response to November 26, 2003 request regarding rat reproductive study
12-3-2003	NDA	FDA Request For Information- list of questions to be discussed during CMC Teleconference on December 8, 2003
12-4-2003	NDA	Response to FDA Request: Proposed agenda, list of Sepracor attendees, and specific questions for CMC teleconference on December 8, 2003
12-8-2003	NDA	CMC Teleconference between FDA and Sepracor
12-16-2003	IND	Submission: Desk copies (5) of Type C Meeting Information Package for the IND
12-16-2003	NDA	Submission: Acknowledgement of Extension of PDUFA date
12-18-2003	NDA	General Correspondence: Pharm/Tox: Summary of rat reproductive study
12-18-2003	NDA	Submission: Summary of Key Findings for Study (rat reproductive study)
12-22-2003	NDA	Submission: Sepracor's Minutes of December 8, 2003 CMC Teleconference
12-23-2003	IND	IND Annual Report: September 1, 2002 through August 31, 2003
12-23-2003	IND	Protocol Amendment: New Investigators (190-050), Revised FDA form 1572 (190-050)
1-6-2004	IND	General Correspondence: Confirmation of location and time for Type C Meeting to be held at FDA on January 14, 2004
1-9-2004	IND	General Correspondence: Postponement of January 14 meeting between FDA and Sepracor
1-16-2004	IND	Protocol Amendment: New Protocol, New Investigators (190-052)
1-21-2004	NDA	General Correspondence: Communication regarding ECAC discussion and decision regarding trade name
1-23-2004	NDA	General Correspondence: inquiry regarding ECAC meeting, if any, and trade name review
1-23-2004	IND	Protocol Amendment: New Investigators (190-050)
1-27-2004	NDA	General Communication: FDA contacted Sepracor regarding transmittal of rat study to Pharm/Tox reviewer
2-2-2004	NDA	Letter to Field Office: Notification of Electronic Submission to CDER – Amendment to a Pending Application: Response to Chemistry Discipline Review Letter Dated October 1, 2003
2-6-2004	NDA	Submission: Response to CMC Discipline Review Letter
2-6-2004	NDA	Response to FDA Request: provided Pharm/Tox Report
2-6-2004	IND	Protocol Amendment: New Protocol, New Investigator (190-054 and 190-055)

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(July 22, 1999 to December 15, 2004)

Date	Type	Activity
2-9-2004	NDA	General Correspondence: Provided desk copy of rat study and related papers
2-11-2004	NDA	Submission: Amendment to NDA: Submission of Audited Draft Report on Rat Reproductive Senescence
2-11-2004	NDA	General Correspondence: Follow-up communications regarding submission of audited draft report for rat reproductive study
2-12-2004	NDA	General Correspondence: Inquiry into unreturned messages by FDA
2-13-2004	IND	Protocol Amendment: Change in Protocol, New Investigators (190-052)
2-17-2004	NDA	General Correspondence: Communications discussing status of pending NDA action
2-20-2004	NDA	General Correspondence: Follow-up to February 17, 2004 conversation - package signed February 19, 2004 and will be delivered to Dr. Temple today, with expected PDUFA letter issuance February 27, 2004
2-23-2004	NDA	General Correspondence: Follow-up communications on delivery of PDUFA action letter package
2-24-2004	IND	Protocol Amendment: Revised FDA form 1572 (190-050)
2-24-2004	NDA	General Correspondence: Action Letter is with the ODE I Division Director and probably will not be processed and signed before February 27, 2004
2-24-2004	NDA	General Correspondence: Sepracor informed FDA of interim Sepracor contact person
2-25-2004	NDA	General Correspondence: FDA informed Sepracor that Action Letter will not go out before the afternoon of February 27, 2004
2-27-2004	NDA	General Correspondence: FDA contacted Sepracor to inform that Action Letter had been signed and was Approvable
2-27-2004	NDA	FDA Letter: Approvable letter for NDA 21-476
3-1-2004	NDA	General Correspondence: Communication to discuss NDA logistics, Request for End of Review Conference, and obtain name of Regulatory Project Manager assigned to the NDA
3-2-2004	NDA	Submission: Acknowledgement of Approvable Letter and Notification of Intent to File an Amendment per 21 CFR 314.110(a)(1)
3-3-2004	IND	General Correspondence: Request for Cancellation of Type C Meeting
3-5-2004	IND	Protocol Amendment: New Protocol, New Investigators (190-054 (48) and 190-055 (42))
3-5-2004	NDA	Submission: Request for End of Review Conference
3-8-2004	NDA	General Correspondence: Communications to discuss Sepracor's upcoming letter of intent to amend the NDA; to confirm FDA's receipt of request for end of review conference, and inform FDA that Sepracor expects conference to be a Type A Meeting
3-9-2004	NDA	Submission: Request for Final Report/Summary from the Executive Carcinogenicity Assessment Committee (Executive CAC)
3-10-2004	IND	Protocol Amendment: Revised Forms FDA 1572 (Protocol 190-029)
3-11-2004	NDA	General Correspondence: Follow-up with FDA concerning the End of Review Conference date and to confirm that FDA has received recent submissions

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(July 22, 1999 to December 15, 2004)

Date	Type	Activity
3-12-2004	IND	Protocol Amendment: New Protocol, New Investigators (190-052 (25))
3-15-2004	NDA	General Correspondence: Inquiry to confirm that NDA will continue to reside at HFD-120 instead of being transferred to DACCADP (HFD-170)
3-16-2004	IND	IND Safety Report: Initial Report (MFR No. 2004SP000040)
3-16-2004	IND	Protocol Amendment: Change in Protocol 190-052 (Amendment 2)
3-18-2004	NDA	General Correspondence: Follow-up on date for end of review conference
3-19-2004	NDA	General Correspondence: FDA Response to submissions dated March 5 & 9, 2004 granting meeting request & scheduling for April 16, 2004.
3-19-2004	NDA	General Correspondence: Sepracor acceptance of April 16, 2004 meeting date, and Request to discuss the nomenclature issue
3-22-2004	NDA	FDA Letter: List of FDA attendees for April 16, 2004 Type B Meeting
3-24-2004	IND	IND Safety Report: Initial Report (MFR No. 2004SP000047, 2004SP000048)
3-29-2004	NDA	General Correspondence: Inquiry as to the number of meeting information packages needed by FDA for the April 16, 2004 Type B Meeting
3-31-2004	NDA	General Correspondence: Regarding list of FDA attendees for the Type B Meeting scheduled for April 16, 2004
3-31-2004	IND	IND Safety Report: Initial Report (MFR No. 2004SP000051)
4-1-2004	NDA	Submission: Type B Meeting Information Package
4-2-2004	NDA	General Correspondence: Request confirmation of FDA's receipt of Type B Meeting Information Package
4-2-2004	NDA	General Correspondence: Submission of Type B Meeting Information Package contained an audio CD not archivable at Central Document Room (No need to resubmit)
4-5-2004	IND	Protocol Amendment: New Investigators (190-054 and 190-055) 16 new Investigators
4-7-2004	NDA	General Correspondence: FDA communication changing meeting date to May 4, 2004, and indicating resubmission will be a Class II resubmission
4-12-2004	NDA	General Correspondence: Sepracor acceptance of new meeting date of May 4, 2004
4-13-2004	IND	IND Safety Report: Initial Report (MFR No. 2004SP000048-FU1)
4-13-2004	IND	Protocol Amendment: New Investigators Revised Forms FDA 1572(Protocol 190-052) 7 new investigators and 12 revised
4-14-2004	NDA	General Correspondence: Follow-up on Nomenclature; Classification of the Resubmission; and End of Review Meeting Logistics
4-20-2004	NDA	General Correspondence: Follow up request for FDA attendees for May 4, 2004 End of Review meeting, and to confirm contact information
4-20-2004	IND	IND Safety Reports: Initial Reports (MFR Nos. 2004SP000067, and 2004SP000068)
4-23-2004	IND	IND Safety Report: Initial Report (MFR No. 2004SP000070)

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(July 22, 1999 to December 15, 2004)

Date	Type	Activity
4-30-2004	IND	IND Safety Reports: Initial Reports (MFR Nos. 2004SP000080, and 2004SP000082)
4-30-2004	NDA	General Correspondence: FDA attendee list for May 4, 2004 meeting
5-3-2004	NDA	General Correspondence: adding two people to the FDA attendee list for May 4, 2004 meeting
5-4-2004	NDA	General Correspondence: New FDA attendee list for May 4, 2004 meeting with new additions
5-4-2004	NDA	End of Review Meeting between FDA and Sepracor
5-6-2004	IND	Protocol Amendment: New Investigators (190-052 and 190-054) 4 new investigators
5-7-2004	IND	IND Safety Reports: Initial Report (MFR No. 2004SP000084), Follow-Up Report (MFR No. 2004SP000067-FU1)
5-10-2004	NDA	General Correspondence: Sepracor sent FDA a complete list of Sepracor attendees to May 4, 2004 meeting, and a request for clarification on the Biopharm Waiver
5-14-2004	NDA	General Correspondence: Communication to FDA relating to open issues
5-14-2004	IND	IND Safety Reports: Initial Report (MFR No. 2004SP000090)
5-17-2004	NDA	General Correspondence: FDA Response regarding the BioPharm bioequivalence waiver
5-20-2004	NDA	Submission: Sponsor Meeting Minutes for May 4, 2004 End of Review meeting
5-21-2004	IND	Protocol Amendment: Revised Forms FDA 1572 (190-029, 190-050)
5-21-2004	IND	IND Safety Reports: Follow-Up Report (MFR No. 2004SP000049-FU1)
5-24-2004	NDA	General Correspondence: Follow-up on proposed new trade name and Sepracor's request for a full waiver of pediatric studies
5-27-2004	IND	IND Safety Reports: 2 follow-up reports (MFR Nos. 2004SP0000067-FU2, and 2004SP0000084-FU1)
6-2-2004	NDA	General Correspondence: FDA Response regarding new trade name and pediatric studies
6-3-2004	NDA	General Correspondence: Inquire as to FDA's receipt of Sepracor's Meeting Minutes, and expected delivery date of FDA's Official Meeting Minutes
6-3-2004	IND	IND Safety Report Initial Report (MFR No. 2004SP000113)
6-4-2004	IND	Protocol Amendment: New Investigators (190-052, 190-054 and 190-055)
6-10-2004	NDA	General Correspondence: Communication informing FDA of resubmission timing, inquiring about meeting minutes, and need to submit draft promotional material in resubmission
6-14-2004	NDA	Submission: Complete response to Action Letter dated February 27, 2004
6-14-2004	IND	IND Safety Report: 7 Day Initial Report (MFR No. 2004SP000129)
6-15-2004	IND	IND Safety Report: 1st follow-up Report (MFR No. 2004SP000082), and 2nd follow-up report (MFR No. 2004SP000084)

Chronology of Significant Activities Regarding IND 58,647 and NDA 21-476 for LUNESTA™ (eszopiclone) Tablets

(July 22, 1999 to December 15, 2004)

Date	Type	Activity
6-16-2004	NDA	Submission: Field Office notification that the Complete response to Action Letter dated February 27, 2004 has been submitted to FDA
6-16-2004	NDA	General Correspondence: Requesting confirmation of FDA Division receipt of resubmission
6-18-2004	IND	IND Safety Reports: Initial Report (MFR No. 2004SP000129), Follow-Up Report (MFR No. 2004SP000068-FU1)
6-22-2004	IND	Protocol Amendment: Revised Forms FDA 1572 (190-050)
6-25-2004	IND	IND Safety Report: Initial Report (MFR. No. 2004SP000130)
6-29-2004	IND	IND Safety Reports: Follow-up Reports (MFR. Nos. 2004SP000070, 2004SP000113, 2004SP000128, and 2004SP000129)
6-29-2004	NDA	General Correspondence: Follow-up on NDA Resubmission and inquire as to 14-day acknowledgement of full response, classification, and new 2nd cycle PDUFA due date for action on resubmission
6-30-2004	IND	Protocol Amendment: New Investigator 190-052
6-30-2004	NDA	General Correspondence: Informing FDA of temporary Sepracor contact person through July 6, 2004
7-9-2004	IND	IND Safety Report: 2004SP000139 – DRAFT Medwatch Form FDA 3500A.
7-12-2004	IND	IND Safety Report: Initial Report (MFR No. 2004SP000139)
7-13-2004	NDA	General Correspondence: Follow-up on NDA resubmission: Confirm receipt of resubmission, completeness, and new action date
7-15-2004	NDA	FDA Letter acknowledging receipt of Sepracor's complete response to the agency's Action Letter of 2/27/2004
7-15-2004	NDA	General Correspondence: Sepracor's acknowledges receipt of FDA Letter concerning the complete response to the agency's Action Letter of 2/27/2004
7-15-2004	IND	IND Safety Report: Initial Report (MFR No. 2004SP000151)
7-20-2004	NDA	General Correspondence: Follow-up with FDA regarding package on new proposed trade name
7-23-2004	NDA	General Correspondence: Follow-up with the Division of Neuropharmacological Drug Products: Status of alternate trade name
7-26-2004	NDA	General Correspondence: Informal update to CMC team leader at FDA providing quick update on Type-II DMF's for racemic zopiclone
7-26-2004	IND	IND Safety Report: Initial Report (MFR No. 2004SP000155), Initial Report (MFR No. 2004SP000156)
8-2-2004	IND	Protocol Amendment: New Investigators (190-050)
8-4-2004	NDA	FDA Request for Information: CMC inquiry
8-5-2004	NDA	Response to FDA Inquiry: CMC Inquiry
8-9-2004	NDA	FDA Request for Information: courtesy desk copies of CMC Submissions from 2/6/2004 and 6/14/2004
8-9-2004	NDA	General Correspondence: confirmation of temporary FDA contact person while primary contact is on vacation

Chronology of Significant Activities Regarding IND 58,647 and NDA 21-476 for LUNESTA™ (eszopiclone) Tablets

(July 22, 1999 to December 15, 2004)

Date	Type	Activity
8-9-2004	NDA	General Correspondence: Review of proposed alternate trade name
8-9-2004	IND	IND Safety Report: Initial Report (MFR No. 2004SP000172)
8-10-2004	IND	Protocol Amendment: Revised Forms FDA 1572 for Protocol 190-052 (44 investigators)
8-10-2004	NDA	General Correspondence: Follow up on primary alternate trade name, and inform FDA of plans for submitting the secondary back-up trade name
8-10-2004	NDA	Submission-Response to FDA Request: providing paper desk copies of CMC Information dated February 6, 2004 and June 14, 2004
8-11-2004	NDA	Submission: Amendment to NDA: Submission of an Alternate Proprietary Trade Name
8-12-2004	NDA	FDA Request for Information: Desk copies (3) of electronic submission of August 11, 2004
8-12-2004	NDA	Submission: Response to FDA Request: providing desk copies (3) of August 11, 2004 Alternate Proprietary Trade Name submission.
8-17-2004	NDA	Response to FDA Request: providing response to August 5, 2004 request
8-18-2004	IND	IND Safety Report: Follow-Up Reports (MFR Nos. 2004SP000113-FU2, and 2004SP000156-FU1)
8-20-2004	NDA	Submission: Amendment to NDA: Response to FDA Request for Information regarding racemic zopiclone
8-23-2004	NDA	Submission: Field Office notification of Electronic Submission to CDER - Amendment to a Pending Application: Response to FDA Request for Information
8-25-2004	IND	IND Safety Report: Follow-Up Reports (MFR Nos. 2004SP000180, and 2004SP000139-FU1), Follow-Up Report (MFR No. 2004SP000090-FU1)
8-26-2004	NDA	Submission: Amendment to NDA: Chemistry, Manufacturing and Controls
8-27-2004	NDA	Submission: Field Office notification of Electronic Submission to CDER - Amendment to NDA: Chemistry, Manufacturing and Controls.
8-31-2004	NDA	General Correspondence: Notice to FDA regarding new contact person for eszopiclone tablets
8-31-2004	IND	General Correspondence: Notification to the FDA of new Sepracor contact person for eszopiclone matters
9-2-2004	IND	Protocol Amendment: New Investigator (1), Revised Form FDA 1572 (1)
9-7-2004	IND	IND Safety Reports: 3 Initial Reports (MFR Nos. 2004SP000188, 2004SP000195, and 2004SP000194)
9-9-2004	NDA	FDA Request for Information: regarding Sepracor's June 14, 2004 submission
9-13-2004	IND	IND Safety Reports: 1 Initial Report (MFR No. 2004SP000199)
9-14-2004	NDA	General Correspondence: Communication confirming FDA will receive information requested September 9, 2004 by no later than October 12, 2004, requesting teleconference to discuss upcoming CMC submission and pending trade name
9-23-2004	NDA	General Correspondence: Provide update on pending submissions to the NDA and status inquiry regarding proposed trade name review

Chronology of Significant Activities Regarding IND 58,647 and NDA 21-476 for LUNESTA™ (eszopiclone) Tablets

(July 22, 1999 to December 15, 2004)

Date	Type	Activity
9-27-2004	NDA	General Correspondence: Notice informing FDA of new Sepracor CMC contact person for the NDA
9-29-2004	NDA	Submission: Amendment to NDA: Chemistry, Manufacturing and Controls
9-30-2004	NDA	General Correspondence: Fax of cover letter for the electronic submission to NDA 21-476 mailed to the Central Document Room on September 29, 2004
9-30-2004	NDA	Submission: Notification of Electronic Submission to CDER – Amendment to NDA: Chemistry, Manufacturing and Controls
9-30-2004	NDA	Submission: Desk copy of CMC Amendment submitted on September 29, 2004
9-30-2004	NDA	Submission: Amendment to NDA: Response to Medical Review Comments dated September 9, 2004
10-1-2004	NDA	General Correspondence: Fax of cover letter for the electronic submission to NDA 21-476 mailed to the Central Document Room on 9/30/2004
10-5-2004	IND	IND Safety Reports: 3 Follow-Up Reports (MFR Nos. 2004SP000172, 2004SP000180, and 2004SP000194)
10-6-2004	IND	IND Safety Report: 1 Initial Report (MFR No. 2004SP000213)
10-7-2004	NDA	General Correspondence: Follow-up with FDA regarding status of proposed trade name review and status of NDA review
10-8-2004	IND	IND Safety Report: Follow-Up Report (MFR No. 2004SP000155-FU1)
10-14-2004	IND	IND Safety Report: Follow-Up Report (MFR No. 2004SP000151-FU1)
10-15-2004	IND	Protocol Amendment: Revised Protocols (190-054 and 190-055)
10-20-2004	IND	IND Safety Reports: Follow-Up Report (MFR No. 2004SP000130), and Follow-Up Report (MFR No. 2004SP000213)
10-21-2004	NDA	General Correspondence: Notice informing FDA of new Sepracor contact person for NDA
10-27-2004	NDA	General Correspondence: Request FDA Division to review timelines
10-28-2004	NDA	General Correspondence: Notice informing FDA of temporary Sepracor contact person for NDA on 10/29/2004
11-1-2004	NDA	General Correspondence: Status inquiry regarding proposed trade name review
11-1-2004	NDA	FDA Request for Information: CMC Question
11-2-2004	NDA	Response to FDA Request: Follow-up to November 1, 2004 CMC question, and request for update on status of Label review
11-4-2004	NDA	General Correspondence: Status inquiry regarding proposed trade name
11-4-2004	IND	IND Annual Report (September 1, 2003, through August 31, 2004)
11-4-2004	IND	General Correspondence: Transfer of IND Responsibility
11-5-2004	IND	IND Safety Report: Follow-Up Report (MFR No. 2004SP000195)
11-5-2004	IND	IND Safety Reports: Follow-Up Report (MFR No. 2004SP000172)
11-5-2004	NDA	General Correspondence: Follow-up with FDA on Sepracor's response to CMC question
11-5-2004	NDA	Submission: Response to FDA Request: Response to FDA CMC Question of November 1, 2004

Chronology of Significant Activities Regarding IND 58,647 and NDA 21-476 for LUNESTA™ (eszopiclone) Tablets

(July 22, 1999 to December 15, 2004)

Date	Type	Activity
11-8-2004	NDA	FDA Request: Provide alternative trade name for eszopiclone tablets
11-8-2004	NDA	FDA Request for Information: Additional CMC questions
11-8-2004	NDA	Response to FDA Request: provide information regarding additional CMC questions
11-8-2004	NDA	Submission: Amendment to NDA: Submission of an Alternate Proprietary Trade Name - Lunesta™
11-8-2004	NDA	General Correspondence: Sepracor Inquiry regarding any further actions Sepracor can take to assist FDA review team
11-9-2004	NDA	FDA Request for Information: CMC - updated stability data tables
11-9-2004	NDA	Response to FDA Request: Communication regarding CMC request for updated stability tables received November 9, 2004
11-9-2004	NDA	Submission: Amendment to NDA: Response to FDA Request for Information
11-9-2004	NDA	General Correspondence: Follow-up on Sepracor's response to CMC question November 1, 2004
11-9-2004	NDA	General Correspondence: Status Inquiry regarding new proposed trade name review, and review of NDA
11-10-2004	NDA	Submission: Notification of Electronic Submission to CDER: Amendment to NDA – Response to FDA Request for Information
11-11-2004	NDA	General Correspondence: Additional Status Inquiry regarding new proposed trade name review, and review of NDA
11-12-2004	NDA	General Correspondence: Communication inquiring if DMETS received alternate trade name submission of November 8, 2004
11-12-2004	NDA	General Correspondence: FDA Response to Sepracor's status inquiries
11-16-2004	NDA	General Correspondence: Follow up on Sepracor's November 8 & 9, 2004 responses to CMC requests
11-16-2004	NDA	Submission: e-mails stability update information for NDA 21-476
11-17-2004	NDA	FDA Request for Information: copies of most recent version of annotated labeling and location of same in previous submission
11-17-2004	NDA	Response to FDA Request: Submitted annotated labeling with track changes
11-17-2004	NDA	Response to FDA Request: provided information on where to find track changes labeling in a previous submission
11-18-2004	NDA	General Correspondence: Communication from FDA informing Sepracor review is underway for most recent trade name submission for eszopiclone tablets
11-18-2004	NDA	FDA Request for Information: Update carton and container labels
11-19-2004	NDA	General Correspondence: Sepracor acknowledges receipt of November 18. 2004 FDA request and inquires as to status of trade name review
11-19-2004	NDA	Submission: Amendment to NDA: Response to FDA Request for Information regarding updated carton and container labels
11-19-2004	NDA	Response to FDA Request: Follow up to request for updated container and carton labeling

Chronology of Significant Activities Regarding IND 58,647 and NDA 21-476 for LUNESTA™ (eszopiclone) Tablets

(July 22, 1999 to December 15, 2004)

Date	Type	Activity
11-22-2004	NDA	Submission: Amendment to NDA: Submission of an Alternate Proprietary Trade Name
11-23-2004	NDA	General Correspondence: Communication informing FDA of two recent submissions
11-23-2004	NDA	General Correspondence: Notice to FDA regarding temporary Sepracor contact person between November 24 and December 3, 2004
11-23-2004	NDA	FDA Communication forwarding proposed draft labeling for eszopiclone tablets, and indication of FDA's availability on November 24,2004 to discuss any questions
11-23-2004	NDA	Response to FDA Request: Providing PDF versions of revised carton and container labeling
11-23-2004	IND	IND Safety Reports: 8 Follow-up reports relating to 190-052 unblinding, 1 Follow-up report relating to 190-050
11-24-2004	NDA	Submission: Amendment to a Pending Application: Response to FDA Request for Information
11-24-2004	NDA	Teleconference with FDA to discuss November 23, 2004 Draft Label
11-24-2004	IND	IND Safety Reports: Follow-up report (MFR No. 2004SP000188-FU1)
11-29-2004	NDA	Revised Label Update
11-29-2004	NDA	Revised Label Update Discussion with Controlled Substance Staff
11-29-2004	NDA	General Correspondence: Notice from FDA regarding temporary FDA contact person from November 30 to December 2, 2004
11-29-2004	NDA	General Correspondence: Exchange of Certificates to Establish Secure e-mail link between Sepracor and FDA
11-29-2004	NDA	Teleconference to discuss Draft label with the Controlled Substance Staff
12-1-2004	NDA	Submission: Notification of Electronic Submission to CDER: Amendment to NDA – Chemistry, Manufacturing and Controls
12-1-2004	NDA	Submission: Revised Draft Labeling
12-1-2004	NDA	Submission: Revised Draft Labeling
12-2-2004	NDA	General Correspondence: Continuing discussions with FDA concerning revised Draft Labeling and DDMAC submissions
12-6-2004	NDA	Notice regarding DMETS assessment and assignment of trade name Lunesta™
12-7-2004	NDA	Teleconference discussing proposed label changes for various sections
12-7-2004	NDA	Responses to FDA Requests: responses to FDA comments and requests of December 7, 2004
12-8-2004	NDA	General Correspondence: FDA transmittal of Draft Labeling as of 12/7/2004
12-8-2004	NDA	General Correspondence: Follow up with FDA on status of DEA notification that eszopiclone has been determined to be Class IV drug
12-8-2004	NDA	Teleconference with FDA regarding proposed Labeling/package insert

Chronology of Significant Activities Regarding IND 58,647 and NDA 21-476 for LUNESTA™ (eszopiclone) Tablets

(July 22, 1999 to December 15, 2004)

Date	Type	Activity
12-8-2004	NDA	General Correspondence: Teleconference regarding clarification of how FDA will communicate approved trade name; FDA agreed to send an e-mail with confirmation of the name Lunesta™
12-8-2004	NDA	General Correspondence: E-Mail Notification from FDA establishing Lunesta™ as approved trade name for eszopiclone
12-8-2004	NDA	General Correspondence: Sepracor Request that FDA provide WORD version of proposed Labeling from the approvable letter.
12-8-2004	NDA	General Correspondence: FDA response to Sepracor request for WORD version of proposed Labeling from approvable letter
12-8-2004	NDA	Submission: Revised draft Labeling as of 12/8/2004 after late afternoon discussions with FDA
12-8-2004	NDA	General Correspondence: Communication to FDA informing FDA of Sepracor's submission of revised draft Label after discussions with FDA
12-8-2004	NDA	Submission: Draft CMC blister labeling and request for FDA input
12-9-2004	NDA	General Correspondence: FDA sending December 6, 2004 draft Controlled Substance section of Labeling to Sepracor
12-10-2004	NDA	General Correspondence: Sepracor providing label comparison file to FDA
12-13-2004	NDA	Submission: Lunesta (eszopiclone) Press Release for DDMAC approval
12-13-2004	NDA	General Correspondence: Request for assignment of a DDMAC reviewer and expedited review of Lunesta (eszopiclone) press release
12-13-2004	NDA	Submission: Revised Draft Label, December 8, 2004
12-13-2004	IND	Protocol Amendment: Revised Forms FDA 1572 for Protocol 190-050 (71 investigators)
12-14-2004	NDA	General Correspondence: FDA transmitting proposed Final Labeling for NDA 21-476
12-14-2004	NDA	Submission: Revised Draft Labeling for NDA 21-476
12-14-2004	NDA	Teleconferences between FDA and Sepracor regarding clarification of Final Labeling wording for NDA 21-476
12-15-2004	IND	Protocol Amendment: New Investigator (1), Revised Forms FDA 1572 (1) for Protocol 190-050
12-15-2004	NDA	General Correspondence: Communication regarding carton label language
12-15-2004	NDA	General Correspondence: Multiple communications regarding latest version of revised carton and container labeling
12-15-2004	NDA	General Correspondence: FDA confirming receipt of latest version of carton label language
12-15-2004	NDA	Submission: Revised Draft Labeling (latest version requested by FDA)
12-15-2004	NDA	FDA Letter of approval for NDA 21-476

EXHIBIT A

Copy of U.S. Patent No. 6,444,673

A SIMPLIFIED METHOD OF EVALUATING DOSE-EFFECT EXPERIMENTS

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The increased emphasis on quantitative biological studies in recent years has resulted in the widespread use of statistical methods for evaluating biological data. Much of this data is of the all-or-none type and, consequently, it is necessary to solve a dose-per cent effect curve. By converting doses to logarithms and per cent effects to probits (1), logits (2), or angles (3), a straight line may be fitted by the method of weighted least squares. From the viewpoint of many biologists, such procedures are not pleasant to contemplate because the data must be converted to units which are meaningless to many and the calculations are difficult, tedious and often quite incomprehensible. It is not surprising therefore that there is widespread use of a variety of approximate methods for solving dose-per cent effect curves. It may be argued that such methods are undesirable because they do not make use of all of the information contained in the data, and are therefore inefficient in a statistical sense. On the other hand, the computations necessary in using efficient methods are often so time-consuming and laborious that the busy experimenter is deterred from using them, and thus loses the advantage of a statistical evaluation of his results. An examination of the various approximate methods, which have been proposed for the solution of dose-effect experiments of the all-or-none type, leads to the conclusion that none of them are entirely satisfactory in combining ease of computation with efficiency and accuracy. In order to appreciate this fact, it is helpful to list the essentials of a satisfactory approximate method for the solution of dose-effect experiments.

(1) The method should give not only the ED_{50} and slope of the curve, but also their confidence limits.

(2) The method should use the data in original units throughout.

(3) Zero and 100 per cent effects should be used effectively.

(4) The method should make it possible to carry out the necessary calculations within 10–15 minutes without a calculating machine, and without resort to logarithms.

(5) The method should recognize heterogeneity when present and give corrected confidence limits in such cases.

(6) The method should facilitate both the comparison of the two curves for parallelism and the computation of relative potency with its confidence limits.

(7) The method should not unduly sacrifice accuracy in favor of simplicity and speed.

The various approximate methods for solving dose-per cent effect curves fail

in varying degrees to meet these requirements. The double integration method (4–7) which uses data in original units and the methods of averages (8–10) which require logarithms provide an estimate of the ED_{50}. With a restricted experimental design, the confidence limits of the ED_{50} can be obtained by the methods of averages, with varying amounts of calculation.

Of the methods which may be considered to approximate that of Bliss (1), one, using the data in original units gives only the confidence limits of the ED_{50} (11); a second, using logarithms gives, in addition to the above, the slope constant, but not its limits (12); and a third, using logarithms and probits gives both parameters and their confidence limits (13). None of the approximate methods use 0 or 100 per cent observations to best effect, or recognize heterogeneity, if present.

The method of Litchfield and Fertig (13), which gives confidence limits of both parameters, appeared to offer the best starting point for developing a revised method which would approach the ideal requirements mentioned above.

In order to revise the above method to conform to the aims listed, three distinct types of problem were involved. The first of these was the problem of using percentages and arithmetic values in a way exactly equivalent to the use of logarithms and probits. Logarithmic-probability paper permits plotting the data in original units but leaves the problem of converting log-probit equations to their arithmetic equivalent.

The result of addition and subtraction of logarithms can be obtained easily by multiplication and division of the numbers themselves. Similarly, the result of multiplying or dividing a logarithm by an arithmetic value can be represented by raising the number corresponding to the logarithm to a power equal to the arithmetic value (or by taking the root in the case of division). Such a calculation cannot be made, however, without recourse to logarithms or to the use of log-log slide rule. Consequently, the second major problem arises, the need for eliminating calculations which require logarithms. In this particular case, a nomograph was constructed for obtaining fractional powers and roots of numbers coming within the scope of the method.

A further complication arises in the case of the product or quotient of two logarithms since this operation cannot be represented at all as a purely arithmetic process. In the two such cases which arise in the revised method, nomographs were constructed to permit solution without recourse to logarithms or a log-log slide rule.

By means of two of the above mentioned nomographs, a simple arithmetic solution of a dose-effect curve can be obtained which is equivalent to the solution by the original method using logarithms and probits. The two solutions are numerically equal but the revised method is more rapid and permits using the data in its original form.

The third type of problem in the revision consisted of finding the means for adding to the method a simple test for heterogeneity or goodness of fit of the line, together with the correction of confidence limits for heterogeneity; a means for effectively using 0 and 100 per cent effects in fitting the line to the data;

and lastly, the means for approximating the confidence limits of doses other than the Median Effective Dose.

The complete method including the necessary tables and nomographs is presented below, together with several examples illustrating its application.

METHOD. The following symbols are used in this method:
K = the number of doses plotted
n = K − 2 = degrees of freedom for $(Chi)^2$
t = value of "Student's" t for p = .05
ED_{50} = Median Effective Dose
S = Slope function
$f_{ED_{50}}$ and f_S = factors for ED_{50} and S, respectively
N′ = total number of animals used between 16 and 84 per cent expected effects
R = the ratio of largest to smallest dose plotted
A = a value derived from S and R
S.R. and P.R. = Slope function Ratio and Potency Ratio
$f_{S.R.}$ and $f_{P.R.}$ = factors for S.R. and P.R., respectively
Unless otherwise indicated all ratios are taken as: larger/smaller value.

PROCEDURE. A. *The data and graph.* 1. List the actual doses used, the number reacting/number tested, and the per cent effects. Do not list more than two consecutive 100 per cent effects at the upper end or more than two consecutive 0 per cent effects at the lower end of the curve.

2. Plot doses against per cent effect on logarithmic-probability paper (No. 3128, Codex Book Co., Inc., Norwood, Mass.) leaving space for but omitting any 0 or 100 per cent effects.

With a transparent straight edge or triangle fit a temporary straight line through the points, particularly those in the region of 40 to 60 per cent effect.

B. *Plotting 0 or 100 per cent effects.* 1. Read and list the expected per cent effect, as indicated by the line drawn, for each dose tested. If the expected value for any dose is less than .01 or greater than 99.99 delete such doses and effects from the list.

2. Using the expected effect record and plot from table 1 a corrected value for each 0 or 100 per cent effect which is listed. Inspect the fit of the line to the completely plotted data. If it is obviously unsatisfactory refit the line and repeat the preceding two steps to obtain a new set of expected and corrected values.

When the line appears to fit satisfactorily, as is almost always the case with the first line, proceed to the $(Chi)^2$ test.

C. *The $(Chi)^2$ test.* 1. List the difference between each observed (or corrected) effect and the corresponding expected effect.

2. Using each difference and the corresponding expected effect read and list the contributions to $(Chi)^2$ from Nomograph No. 1. (A straight edge connecting a value on the expected per cent scale with a value on the difference scale, will indicate at the point of intersection of the $(Chi)^2$ scale, the contribution to $(Chi)^2$.)

3. Total the contributions to $(Chi)^2$ and multiply by the average number of animals per dose, i.e., the total number of animals/K, the number of doses. This is the $(Chi)^2$ of the line. The degrees of freedom are two less than the number of doses plotted, i.e., n = K − 2.

4. If the $(Chi)^2$ of the line is less than the value of $(Chi)^2$ given in table 2 for n degrees of freedom, the data are not significantly heterogeneous, i.e., the line is a good fit. If the $(Chi)^2$ of the curve exceeds the value of $(Chi)^2$ given in table 2, the data are significantly heterogeneous and the line is not a good fit. (If the $(Chi)^2$ of the line cannot be reduced below the permissible $(Chi)^2$ by refitting the line, the value of t in table 2 for n degrees of freedom should be noted.)

D. *The ED_{50} and $f_{ED_{50}}$.* 1. Read from the line on the graph the dose for 16, 50, and 84 per cent effects (ED_{16}, ED_{50} and ED_{84}).

2. Calculate the slope function, S, as:

$$S = \frac{ED_{84}/ED_{50} + ED_{50}/ED_{16}}{2}.$$

3. Obtain from the data tabulation, N′, the total number of animals tested at those doses whose *expected effects* were *between* 16 and 84 per cent.

4. Calculate the exponent in the expression:

$$f_{ED_{16}} = S^{2.77/\sqrt{N'}} = S^{\text{exponent}}.$$

To carry out this step, obtain first the $\sqrt{N'}$ from a square root table, or with a slide rule, or by means of Nomograph No. 2. Then solve $2.77/\sqrt{N'}$ = exponent. Next, using this exponent and the value of S, read the $f_{ED_{16}}$ on the center scale of Nomograph No. 2 by laying a straight edge across the correct scale values.

5. Calculate the confidence limits of the ED_{50} as:

$$\left.\begin{aligned} ED_{50} \times f_{ED_{16}} &= \text{upper} \\ ED_{50}/f_{ED_{16}} &= \text{lower} \end{aligned}\right\} \text{limit for 19/20 probability.}$$

E. *S and f_S*

1. Calculate the dosage range as a ratio, as follows:

$$R = \text{largest/smallest dose plotted}$$

2. Using this value of R and that of S (from step D2), read the value designated as A from Nomograph No. 3 by laying a straight edge across the correct scale values.

3. Solve for the exponent in the following expression, using K from step C3 and $\sqrt{N'}$ from step D4.

$$f_S = A^{10(K-1)/K\sqrt{N'}} = A^{\text{exponent}}$$

Then with this exponent and the value of A, read f_S from Nomograph No. 2.

4. Calculate the confidence limits of S as:

$$\left.\begin{aligned} S \times f_S &= \text{upper} \\ S/f_S &= \text{lower} \end{aligned}\right\} \text{limit for 19/20 probability.}$$

F. *The factors for significantly heterogeneous data.* When the $(Chi)^2$ test indicates significant heterogeneity the value of t from table 2 is noted and the formulas below are used for the factors instead of those in steps D4 and E3. The procedure for solution consists of solving first for the values of the exponent and then with the value of S from step D2 and that of A from step E2, the factors are read from Nomograph No. 2.

1. $f_{ED_{16}} = S^{1.4t\sqrt{(Chi)^2/nN'}} = S^{\text{exponent}}$

2. $f_S = A^{[5.1t(K-1)\sqrt{(Chi)^2/nN'}]/K} = A^{\text{exponent}}$

G. *The test for parallelism of two lines and the estimate of relative potency.* The following values which represent the parameters and factors of a dose-per cent effect line are to be compared to a similar set of values for a second line:

$$\left.\begin{aligned} &ED_{16} \text{ and } f_{ED_{16}} \\ &S \text{ and } f_S \end{aligned}\right\} \text{for each line.}$$

1. The test for parallelism: the slope function ratio, S.R.

(a) Calculate: S.R. = S_1/S_2 where S_1 is the larger value.

(b) Using f_{S_1} and f_{S_2} read $f_{S.R.}$ from the center scale of Nomograph No. 4 by laying a straight edge across the correct scale values.

(c) If the value of S.R. exceeds the value of $f_{S.R.}$ the curves deviate significantly (19/20 probability) from parallelism. If S.R. is less than $f_{S.R.}$ the curves may be considered parallel within experimental error and the potency ratio may then be computed as follows:

2. The Potency Ratio, P.R.
(a) Calculate: P.R. = ED_{50_1}/ED_{50_2} where ED_{50_1} is the larger value.
(b) Using $f_{ED_{50_1}}$ and $f_{ED_{50_2}}$ read $f_{P.R.}$ from the center scale of Nomograph No. 4.
(c) The value of P.R. must exceed the value of $f_{P.R.}$ for the two substances being compared to differ significantly in potency.
3. The confidence limits of the slope and the potency ratio are given by:

$$\text{(a)}\ \left.\begin{matrix}\text{S.R.} \times f_{S.R.} = \text{upper} \\ \text{S.R.}/f_{S.R.} = \text{lower}\end{matrix}\right\} \text{limit for 19/20 probability.}$$

$$\text{(b)}\ \left.\begin{matrix}\text{P.R.} \times f_{P.R.} = \text{upper} \\ \text{P.R.}/f_{P.R.} = \text{lower}\end{matrix}\right\} \text{limit for 19/20 probability.}$$

The following example illustrates the use of the method. On the work sheet below the various steps are indicated by A1–E4. The graph (fig. 1) corresponds to step A2.

Solution of the Dose-Effect Curve of Tagathen (Chlorothen Citrate) against Histamine

A1 DOSE	A1 ALIVE/TESTED	A1 OBSERVED % ALIVE	B1 EXPECTED % ALIVE		C1 OBSERVED MINUS EXPECTED	C2 (NOMOGRAPH NO. 1) CONTRIBUTION TO $(CHI)^2$
mgm./kgm.						
1.0	8/8	100 (99.5) B2(table 1)	98.6		0.9	0.006
0.5	7/8	88	90.5		2.5	0.007
0.25	4/8	50	67	D3	17.0	0.110
0.125	4/8	50	34		16.0	0.105
0.0625	1/8	12	9.5		2.5	0.007

C3 Total animals = 40; Number of Doses, K = 5; Animals/Dose = 40/5 = 8 — Total 0.235; $(Chi)^2 = 0.235 \times 8 = 1.88$; Degrees of Freedom, n = K − 2 = 3

C4 $(Chi)^2$ from table 2 for n of 3 = 7.82. 1.88 is less than 7.82, therefore the data are not significantly heterogeneous.

D1 ED_{84} mgm./kgm. = 0.390
ED_{50} mgm./kgm. = 0.175
ED_{16} mgm./kgm. = 0.080

D2
$$S = \frac{ED_{84}/ED_{50} + ED_{50}/ED_{16}}{2}$$
$$S = \frac{0.390/0.175 + 0.175/0.080}{2} = 2.2$$

D3 (Note Bold face limits above) N′ = 16

D4 $f_{ED_{50}} = (S)^{2.77/\sqrt{N'}} = 2.2^{2.77/\sqrt{16}} = (2.2)^{0.69} = 1.72$ (from Nomograph No. 2)

D5 $ED_{50} \times f_{ED_{50}} = 0.175 \times 1.72 = 0.30$ mgm./kgm.
$ED_{50} / f_{ED_{50}} = 0.175 / 1.72 = 0.10$ mgm./kgm.
ED_{50} and 19/20 confidence limits: 0.18 (0.10 to 0.30) mgm./kgm.

E1 R = largest/smallest dose = 1.0/0.0625 = 16

E2 A = 1.27 (from Nomograph No. 3, using S = 2.2 and R = 16)

E3 $f_S = (A)^{10(K-1)/K\sqrt{N'}} = (1.27)^{10\times 4/5\sqrt{16}} = (1.27)^{2.0} = 1.60$ (from Nomograph No. 2)

E4 $S \times f_S = 2.2 \times 1.6 = 3.5$
$S / f_S = 2.2 / 1.6 = 1.4$
S and 19/20 confidence limits: 2.2 (1.4 to 3.5)

In practice the work sheet can be greatly condensed in comparison to the example by omission of the various step symbols, formulae and by recording on the graph the figures

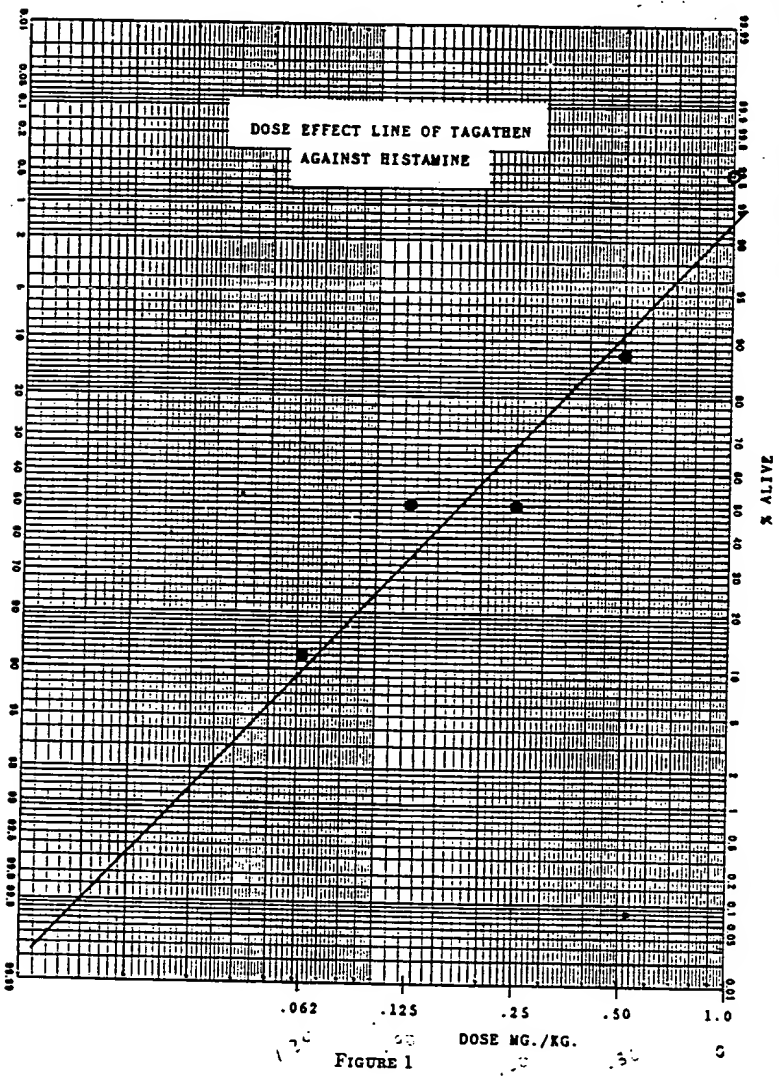
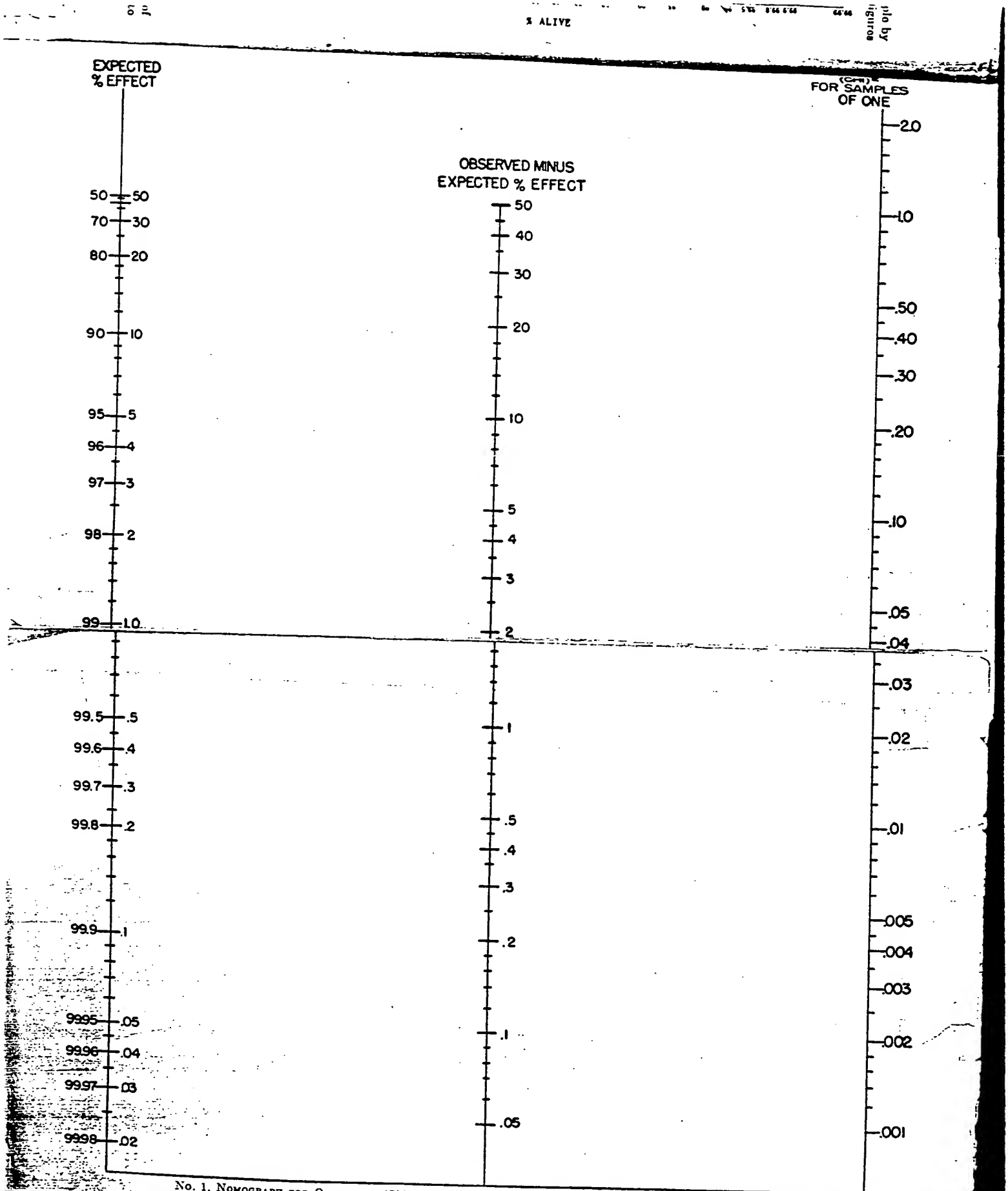


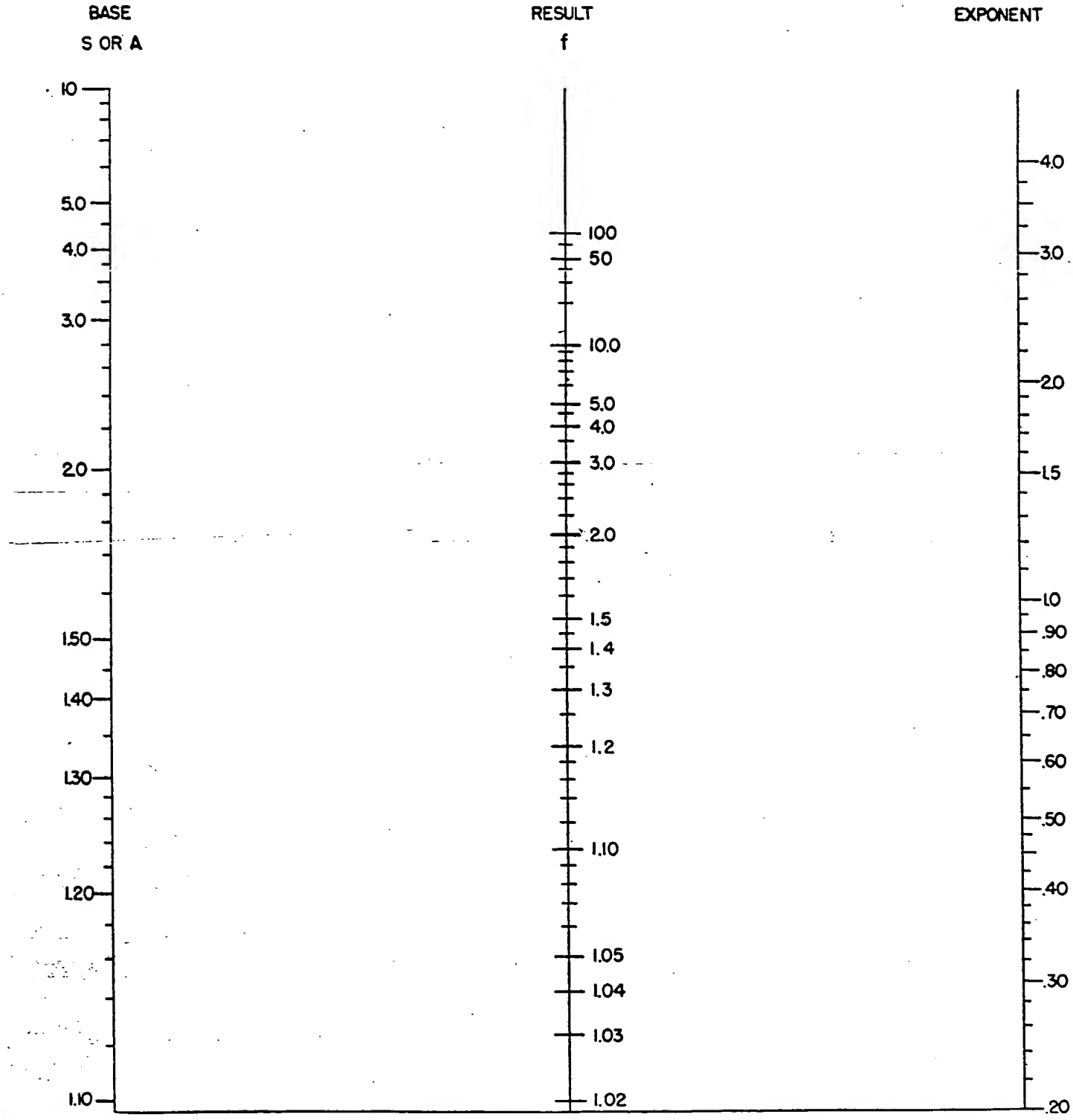
FIGURE 1

used in the calculations. In the same experiment in which the antihistamine activity of Tagathen was studied, tripelennamine (Pyribenzamine) was tested similarly in order to

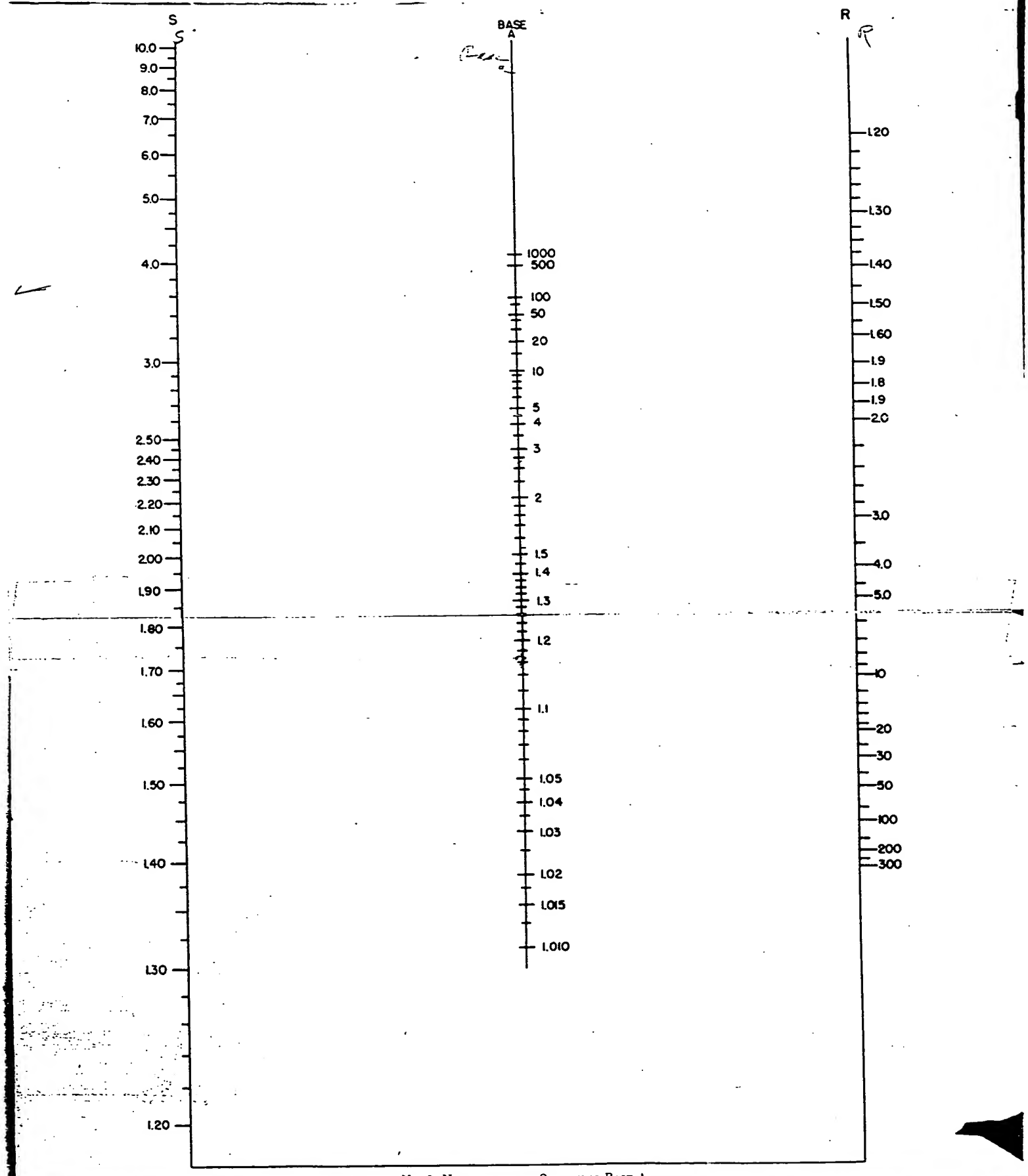
EXPECTED % EFFECT



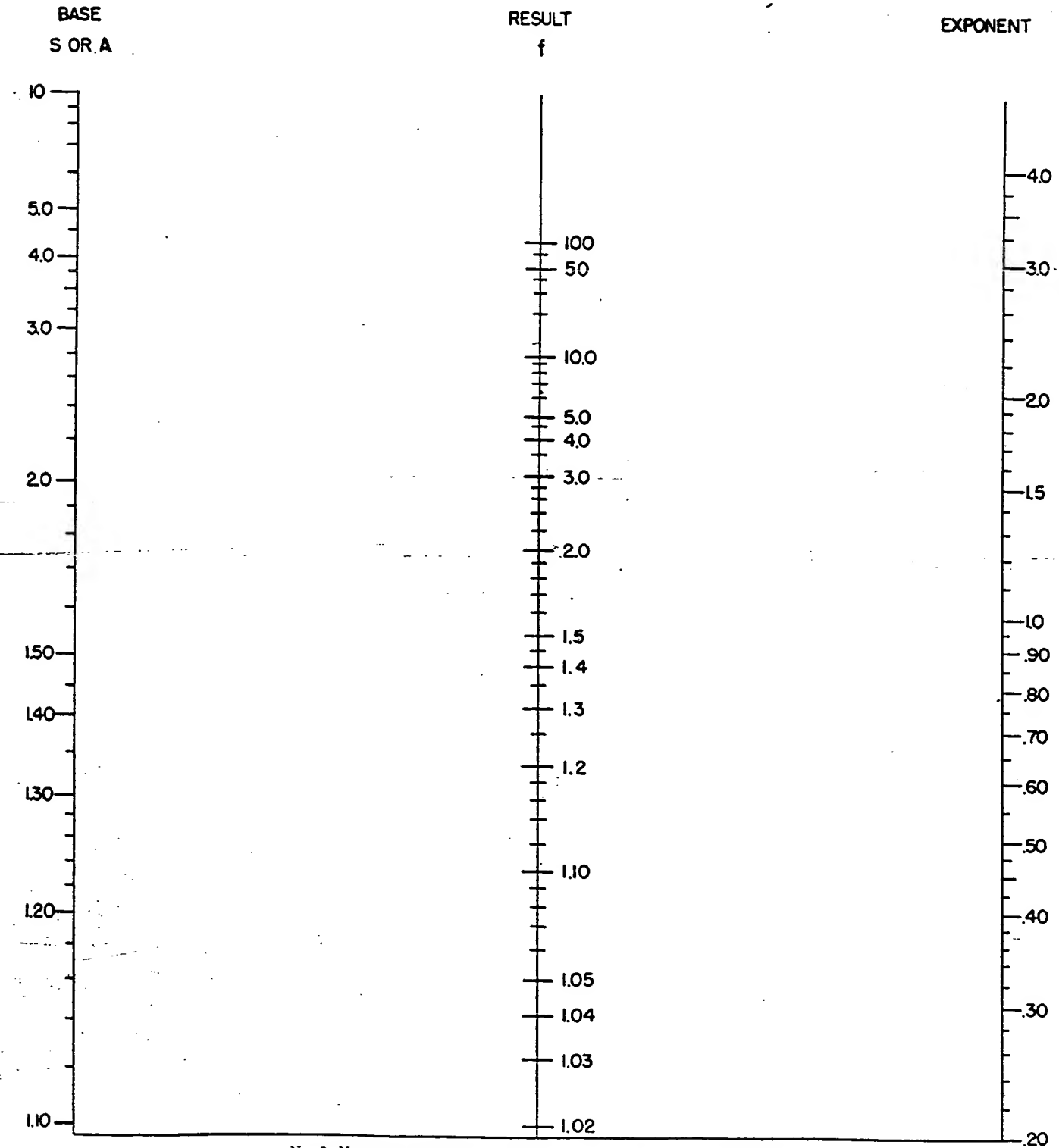
No. 1. Nomograph for Obtaining (Chi)^2 from Expected % Effect and Observed-Expected % Effect



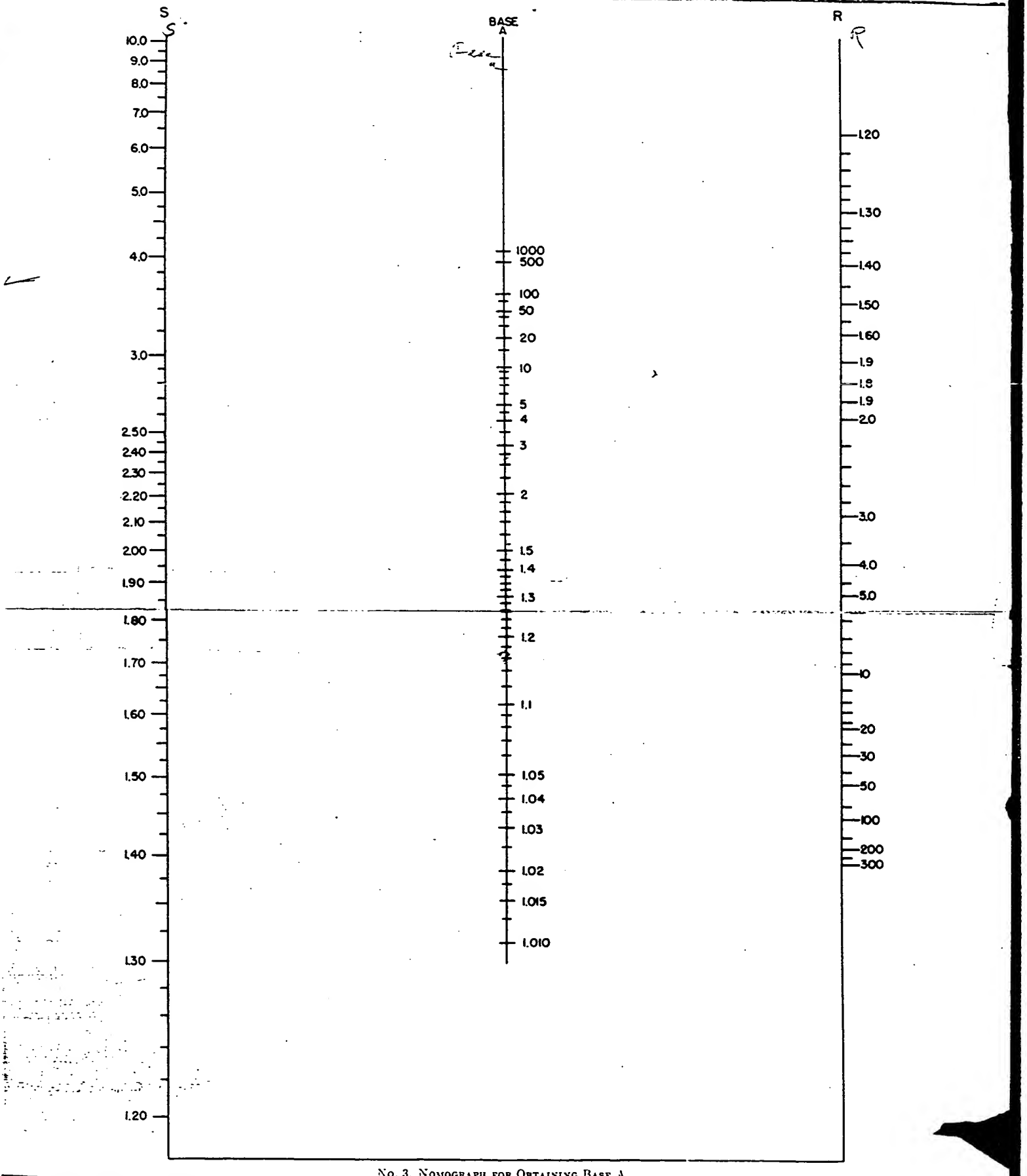
No. 2. Nomograph for Raising Base S or A to a Fractional Exponent



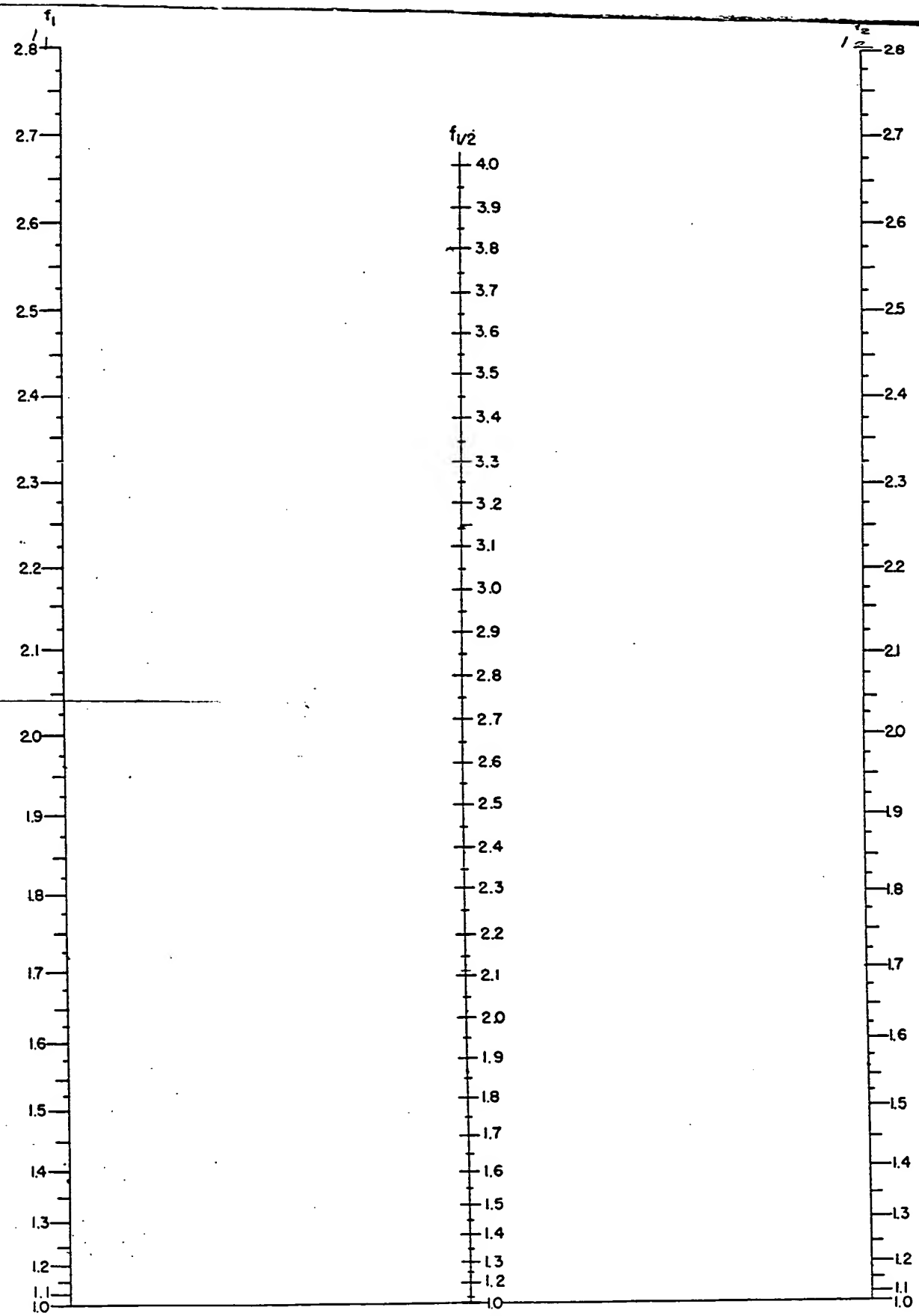
No. 3. Nomograph for Obtaining Base A



No. 2. Nomograph for Raising Base S or A to a Fractional Exponent



No. 3. Nomograph for Obtaining Base A



No. 4. Nomograph for Obtaining the Factor for the 19/20 Error of the Ratio of two Values

determine the potency ratio of the two drugs. The solution of the dose effect curve of Pyribenzamine illustrates the combined work sheet and graph.

The parameters and factors of the two dose effect curves are summarized below.

	Tagathen	*Pyribenzamine*
ED_{50}	0.18 mgm./kgm.	0.60 mgm./kgm.
$f_{ED_{50}}$	1.72	1.60
S	2.20	2.34
f_S	1.60	1.57

The curves are tested for parallelism and the potency ratio obtained as follows:

G1 The slope ratio, S.R. $= S_1/S_2 = 2.34/2.20 = 1.06$

$f_{S.R.} = 1.92$ (from Nomograph No. 4, using the two f_S values)

S.R. of 1.06 is less than $f_{S.R.}$ of 1.92, therefore, the deviation from parallelism is not significant.

G2 The potency ratio, P.R. $= ED_{50_1}/ED_{50_2} = 0.60/0.18 = 3.3$

$f_{P.R.} = 2.05$ (from Nomograph No. 4, using the two $f_{ED_{50}}$ values)

P.R. of 3.3 exceeds $f_{P.R.}$ of 2.05, therefore, the two drugs differ significantly in potency.

G3 Confidence limits of S.R. and P.R.

S.R. $\times f_{S.R.} = 1.06 \times 1.92 = 2.03$

S.R./$f_{S.R.} = 1.06/1.92 = 0.55$

The slope ratio, S.R., and 19/20 confidence limits: 1.06 (0.55 to 2.03).

P.R. $\times f_{P.R.} = 3.3 \times 2.05 = 6.75$

P.R./$f_{P.R.} = 3.3/2.05 = 1.60$

The potency ratio, P.R., and 19/20 confidence limits: 3.3 (1.6 to 6.8). Tagathen was significantly more active than Pyribenzamine and for confidence limits of 19/20, its relative activity lies between 1.6 and 6.8 times that of Pyribenzamine.

Occasionally the experimenter is interested in a dose other than the ED_{50}; for example, he may wish to know the ED_{90} and its confidence limits. The dose for any desired per cent effect, Y, can be read from the graph. The 19/20 confidence limits of this dose, ED_Y, can be approximated by increasing the value of $f_{ED_{50}}$ by an amount determined by the value of fs and X, (the *deviation* in standard deviation units, of Y from 50 per cent). Values of X for common values of Y are given in table 3.

The procedure for obtaining the 19/20 confidence limits of ED_Y is as follows:

1. Obtain $(f_S)^X$ using Nomograph No. 2 to raise the base, f_S (from step E3) to the exponent X (from table 3).

2. Obtain f_{ED_Y} from center scale of Nomograph No. 4, using the value obtained for $(f_S)^X$ and the value of $f_{ED_{50}}$ (from step D4). If the scale limits of the nomograph are exceeded, the confidence limits are likely to be so wide that the ED_Y value is rather meaningless. The confidence limits are obtained in the usual way using the f_{ED_Y}.

As an example of this procedure applied to the Tagathen line, the confidence limits of $ED_{90} = 0.48$ mgm./kgm. are obtained as follows:

1. $(f_S)^X = 1.60)^{1.3} = 1.85$ (from Nomograph No. 2 and table 3).

2. $f_{ED_{90}} = 2.25$ (from Nomograph No. 4, using $(f_S)^X = 1.85$ and $f_{ED_{50}} = 1.72$).

ED_{90} and 19/20 confidence limits = 0.48 (0.21 to 1.08) mgm./kgm.

DISCUSSION. The method presented fulfills, for the most part, the aims of a satisfactory approximate method. At the risk of making the method appear rather complex the instructions have been made as complete as possible. We have found that these instructions in the hands of an inexperienced person permit a complete solution of data, such as that in fig. 1, to be obtained in 20 to 30 minutes. An experienced person on the other hand requires less than half

this time. Although the accuracy of this method has not been examined, it cannot be less than that of the Litchfield-Fertig method which has been shown

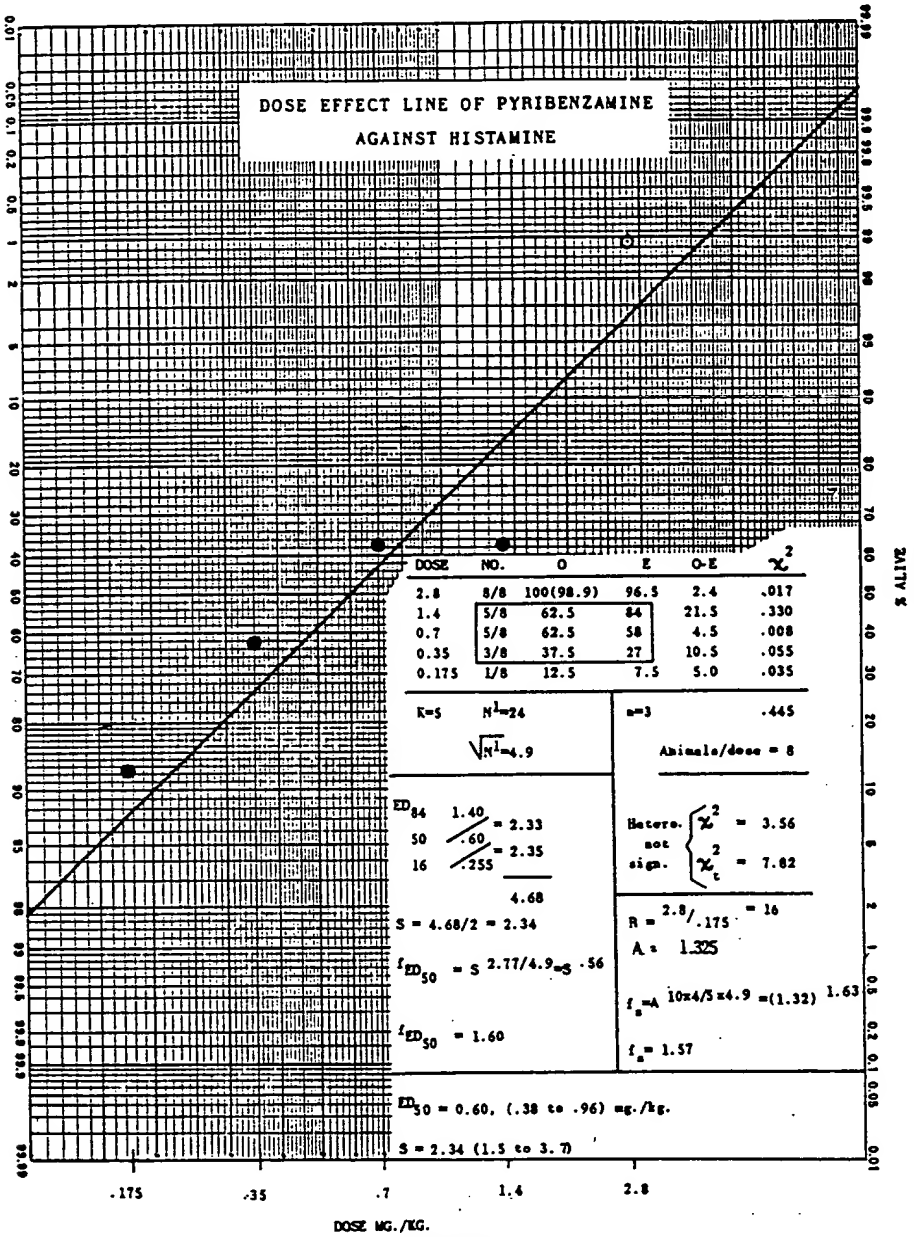


FIGURE 2

to be satisfactory for all ordinary purposes. The new method in some respects is undoubtedly more accurate since not only can a poorly fitted line be detected and improved but also significant heterogeneity, if present, will be found.

TABLE 1

Corrected Values of 0 or 100 per cent Effect (Body of Table) Corresponding to Expected Values (Margins)*

EXPECTED	0	1	2	3	4	5	6	7	8	9
0	—	0.3	0.7	1.0	1.3	1.6	2.0	2.3	2.6	2.9
10	3.2	3.5	3.8	4.1	4.4	4.7	4.9	5.2	5.5	5.7
20	6.0	6.2	6.5	6.7	7.0	7.2	7.4	7.6	7.8	8.1
30	8.3	8.4	8.6	8.8	9.0	9.2	9.3	9.4	9.6	9.8
40	9.9	10.0	10.1	10.2	10.3	10.3	10.4	10.4	10.4	10.5
50	—	89.5	89.6	89.6	89.6	89.7	89.7	89.8	89.9	90.0
60	90.1	90.2	90.4	90.5	90.7	90.8	91.0	91.2	91.4	91.6
70	91.7	91.9	92.2	92.4	92.6	92.8	93.0	93.3	93.5	93.8
80	94.0	94.3	94.5	94.8	95.1	95.3	95.6	95.9	96.2	96.5
90	96.8	97.1	97.4	97.7	98.0	98.4	98.7	99.0	99.3	99.7

* These values are derived from the maximal and minimal corrected probits of Bliss (1).

TABLE 2

Values of t and (Chi)² for p = .05*

DEGREES OF FREEDOM	t	$(\text{CHI})_2$
1	12.7	3.84
2	4.3	5.99
3	3.18	7.82
4	2.78	9.49
5	2.57	11.1
6	2.45	12.6
7	2.36	14.1
8	2.31	15.5
9	2.26	16.9
10	2.23	18.3

* Values of "students" t and (Chi)² for p = .05 are the same as may be found in more extensive tables such as those in (17).

TABLE 3*

% EFFECT, Y	X
16 or 84	1.00
10 or 90	1.30
5 or 95	1.65
2 or 98	2.05
1 or 99	2.35

* Other values of X may be obtained from any table relating deviations and areas of the normal curve, such as (17).

All approximate methods without exception have one or more weak points. The inadequacy of these methods becomes evident when they are applied to an

unbalanced or truncated set of data. Thus in the more exact method of Bliss (1), the confidence limits are corrected for the deviation (caused by unbalance), of the mean probit from 5.0. Due to the nature of the weighting coefficients this correction is of little significance unless the degree of truncation is rather large. Thus, in the case of any approximate method, the confidence limits will tend to be underestimated when the maximum observed effect is 70 per cent or less. This weakness common to all such methods is usually overlooked and the experimenter should avoid applying an approximate method to very poorly balanced experiments. In such cases repetition of the experiment or the use of the more exact procedure of Bliss (1) is indicated. It cannot be sufficiently emphasized, however, that a statistical method is in no way a substitute for a good experiment.

The name slope function has been applied to S, the antilogarithm of the quantity designated as s in Bliss' notation, or λ in Gaddums notation (18), where s or λ is the standard deviation of the logarithms of the individual effective doses. Our choice was based on the use of S for purposes customarily served by the slope constant b, and we are not aware of any existing designation for the antilogarithm of the standard deviation.

Since the dose-per cent effect curve is encountered so frequently in biological and occasionally even in non-biological fields, this rapid approximate method should be of help to the many individuals who have not the time, desire nor facilities for complex mathematical treatment of this kind of data.

SUMMARY

1. A rapid graphic method for approximating the Median Effective Dose and the Slope of dose-per cent effect curves is presented. Confidence limits of both of these parameters for 19/20 probability are given by the method. In addition, confidence limits for any other probability or for a dose other than the Median Effective Dose are readily estimated.

2. The data are used throughout the method in their original form without transformation to logarithms and probits.

3. An effective means for plotting and using 0 and 100 per cent effects is provided.

4. The calculations have been simplified by means of nomographs to the extent that a slide rule is a convenience but not a necessity.

5. A simple means is provided for detecting a poorly fitted line or significantly heterogeneous data. In the former case, the line may be refitted; in the latter, the confidence limits are corrected for the degree of heterogeneity.

6. The method provides means for the rapid test of parallelism of two curves and easy computation of relative potency with its confidence limits.

7. Although the method is rapid (10–15 minutes), its accuracy is commensurate with the nature of dose-per cent effect data.

APPENDIX

A. *Source or derivation of formulae used in the method.* The revised method uses in modified form: (1) The approximations developed by Litchfield and Fertig (13) for obtaining confidence limits of the parameters of a dose-per cent effect curve, and (2) the method for $(Chi)^2$ proposed by Wilcoxon and McCallan (14). The corrections used in the event of heterogeneity, the method for using 0 and 100 per cent effects, and the test for significant differences between values are derived from conventional procedures (1). The formula for obtaining approximate confidence limits of doses giving per cent effects other than 50 per cent is derived from that for the variance of the log ED_{50} as given by Bliss (1).

The following table shows some of the relationships between formulae used in the revised method and their equivalents after transformation to the logarithm-probit system.

Arithmetic method	*Log-probit method*
ED_{50}	$\log ED_{50}$
$f_{ED_{50}}$	$1.96SE_{\log ED_{50}}$
S	$\log S$ or s or $1/b$
f_S	$1.96S.E._s$ or $1.96s^2S.E._b$
A/B	$\log A - \log B$
$f_{A/B}$	$1.96\sqrt{(S.E._{\log A})^2 + (S.E._{\log B})^2}$
$f_{ED_{Y\%}}$	$1.96\sqrt{S.E.^2_{\log ED_{50}} + [S.E._s(y - 5.0)]^2}$
Corrected effect for 0 or 100 per cent	Maximal or minimal corrected probit

B. *The parameters and confidence limits of a dose per cent effect line on logarithmic-probability paper.* 1. *The median effective dose: ED_{50}.* This is the dose indicated by the line to cause 50 per cent of the animals or items to react or not, to live or die, to be positive or negative, to fit into a category or not, etc. Dose is used in the abstract sense and may be dose, time, size, distance, etc.

2. *The slope function of the line: S.* This is the fold change in dose required to produce a unit standard deviation change in response along the line. Thus:

$$S = \text{antilog of: } 1/b,\ s,\ \text{or } \frac{X_1 - X_2}{Y_1 - Y_2}$$

where b and s are, respectively, the slope constant and standard deviation of a line relating log dose X, and probit per cent effect Y. Since s is actually the difference between two particular log doses, its antilog, the slope function S, is the ratio of the arithmetic value of those doses.

3. *The factor of $f_{ED_{50}}$ for obtaining 19/20 confidence limits of the ED_{50}.* This factor, using the notation of Litchfield and Fertig, is derived as follows:

$$S.E._{\log ED_{50}} = \frac{s}{\sqrt{N'/2}} \tag{1}$$

where s is the difference between two log doses whose *expected* effects, *as indicated by the line*, differ by 1.0 probit and N′ is the total number of animals or items tested between the log dose limits corresponding to *expected* probits 4.0 and 6.0.

Multiplying (1) by 1.96, simplifying and taking the antilog gives:

$$f_{ED_{50}} = S^{2.77/\sqrt{N'}} \tag{2}$$

where S = antilog s and N′ is now the total number of animals or items tested between arithmetic dose limits corresponding to *expected* 16 and 84 per cent effect.

The slope function S can be obtained from the line on logarithmic-probability paper by any of the following expressions but (3) is preferable:

$$S = ED_{84}/ED_{50} \quad \text{or} \quad ED_{68}/ED_{31} \quad \text{or} \quad ED_{50}/ED_{16}$$

$$S = \frac{ED_{84}/ED_{50} + ED_{50}/ED_{16}}{2} \tag{3}$$

The factor for the ED_{50} can be reduced to the expression:

$$f_{ED_{50}} = S^{exp.}$$

where the exponent is $2.77/\sqrt{N'}$. The value of $f_{ED_{50}}$ can then be read from the fractional power Nomograph No. 2.

4. *The factor f_S for obtaining limits for 19/20 probability of the slope function S.* This factor is derived from the approximation of Litchfield and Fertig to the standard error of the slope constant, b.

Since:

$$s = 1/b \text{ and } s^2 = 1/b^2$$

By differentiation:

$$ds = -b^{-2}\,db = -(1/b^2)\,db$$

Then by substitution:

$$ds = -s^2\,db \text{ or}$$

$$S.E._s = s^2 S.E._b \tag{4}$$

The minus sign can be dropped because it merely signifies the reciprocal relation between s and b.

The approximation to the standard error of b for limits for 19/20 probability is given by:

$$1.96 S.E._b = \frac{7.85}{\sqrt{N'/2}\,LK/K-1}$$

where L and K refer, respectively, to the logarithmic dosage range of the experiment and the number of doses tested. N′ is the same as defined above. This can be simplified to give:

$$1.96 S.E._b = \frac{11.1(K-1)}{LK\sqrt{N'}} \tag{5}$$

By substitution of (5) in equation (4): and rewriting:

$$1.96 S.E._s = \frac{11.1\,s^2(K-1)}{LK\sqrt{N'}} = \frac{1.1\,s^2}{L} \times \frac{10(K-1)}{K\sqrt{N'}}$$

Let: $A = \text{antilog } \frac{1.1\,s^2}{L}$,

$R = \text{antilog } L$

and, since $S = \text{antilog } s$,

then $A = \text{antilog } \frac{1.1(\log S)^2}{\log R}$ (6)

and $f_S = \text{antilog } 1.96 S.E._s = A^{10(K-1)/K\sqrt{N'}} = A^{s^{exp.}}$ (7)

The value of S is known from equation (3) and the value of R is given by the ratio: R = largest/smallest dose plotted. In order to eliminate the use of logarithms, Nomograph No. 3, having scale values in units of S and R, was constructed to solve equation (6) to give the value of A. K is the number of doses plotted and N′ has already been defined. The factor for S can then be read from Nomograph No. 2 using the value of A and its exponent.

The confidence limits for the parameters ED_{50} and S are obtained by using the factors $f_{ED_{50}}$ and f_S as follows:

$$\left.\begin{aligned}\text{Parameter} \times f &= \text{upper}\\ \text{Parameter}/f &= \text{lower}\end{aligned}\right\} \text{limit for 19/20 probability.}$$

C. *Additions to the basic method.* 1. *Use of 0 and 100 per cent effects.* Fisher (cited by Bliss, 1) has shown that the most likely value for 0 or 100 per cent effects is the minimal or maximal corrected probit, the exact value of which is determined by the expected probit obtained from the line on the log dose probit graph. The equivalent procedure for 0 or 100 per cent effect in the case of plotting on logarithmic probability paper is made possible by means of a table relating the expected per cent effect, indicated by the line, to the minimal or maximal corrected per cent effect. These corrected values have been interpolated and converted to percentages from the original table of corrected probits (1).

The procedure for using 0 or 100 per cent effects consists of: (a) plotting the data on logarithmic probability paper, omitting 0 or 100 per cent effects, and fitting a temporary line with transparent straight edge or triangle; (b) reading the expected per cent effect indicated by the straight edge at doses where 0 or 100 per cent effect was observed; (c) converting the expected per cent effect to a corrected value by means of table 1 and plotting this corrected value; (d) drawing a line through the completely plotted data.

2. *Recognition of heterogeneous data: Test of the line for "Goodness of Fit."* The nomographic calculation of $(Chi)^2$, previously reported by Wilcoxon and McCallan (14), was modified slightly and incorporated into the revised method. In this portion of the procedure, the expected per cent effects from the line are listed opposite the observed per cent effect and a list of differences between observed and expected per cent effects made. For each set of a difference and the corresponding expected per cent effect, a $(Chi)^2$ value based on one animal or item is read from Nomograph No. 1. The total of these $(Chi)^2$ values multiplied by the average number of animals or items per dose is the $(Chi)^2$ of the dose-effect line. The degrees of freedom, n, are two less than the number of points plotted, i.e., $n = K - 2$. By comparison of this to the value of $(Chi)^2$ for probability of .05 and n degrees of freedom, significant heterogeneity can be recognized. In the event of significant heterogeneity a better fitting line can often be drawn, and if not, the equations for the factors of the parameters are modified to include this additional variation. For this modification the value of "students" t for a probability of .05 and n degrees of freedom must be used. For convenience in using the method, the important values of "students" t, and $(Chi)^2$ for $p = .05$ and various degrees of freedom are given in table 2. Other values may be found in more extensive tables such as are given by Snedecor (15).

When significant heterogeneity is found the factors of the parameters are obtained by the following equations, whose nomographic solution is the same, however, as described above.

For heterogeneous data

$$f_{ED_{50}} = S^{1.4t\sqrt{(Chi)^2/nN'}} = S^{exp.} \tag{8}$$

$$f_S = A^{[5.1t(K-1)\sqrt{(Chi)^2/nN'}]/K} = A^{exp.} \tag{9}$$

All symbols have the same significance as noted above.

The change which has been made in the exponents of both of the factors in order to correct for heterogeneity is the conventional multiplier (16) while the value of "t" replaces the 1.96 which was previously introduced into the exponent and must now be divided out again. Thus, for limits for 19/20 probability,

$$(Exp._{homogeneous}) \times \frac{t\sqrt{(Chi)^2/n}}{1.96} = Exp._{heterogeneous}.$$

Nomograph No. 1 computes $(Chi)^2$ for a single item as:

$$(Chi)^2 = \frac{(\text{Observed} - \text{expected per cent effect})^2}{(\text{Expected effect})(100 - \text{expected effect})} \tag{10}$$

which is derived from the expression used by Wilcoxon and McCallan (14) for nomographic solution of $(Chi)^2$ for 100 items.

3. *Comparison of two dose-effect curves: The test for parallelism and the ratio of potencies.* In the method of Bliss (1) and the approximate method of Litchfield and Fertig (13), the significance of differences between the parameters of two curves was tested by computing the standard error of the difference as:

$$S.E._{Diff.} = \sqrt{(S.E._1)^2+(S.E._2)^2}$$

In the revised method, the equivalent arithmetic procedure is used; that is, in place of a difference between two logarithmic quantities, the ratio of the quantities themselves is used. Furthermore, in place of the standard error of a log quantity, the factor of the quantity itself is used since, as already noted, the factor is the antilog of the Standard Error.

Thus, for limits for 19/20 probability, in place of $1.96 S.E._{Diff.} = \sqrt{(\log f_1)^2 + (\log f_2)^2}$, the following equation is used:

$$f_{ratio} = \text{antilog } \sqrt{(\log f_1)^2 + (\log f_2)^2} \qquad (11)$$

To eliminate logarithms, Nomograph No. 4 was constructed having scale values in units of f_1 and f_2 which, if connected by a straight edge, permit reading f_{ratio} on the intersected center scale. The procedure is the same for both the factor of the ratio of the slope functions S_1/S_2 or the ratio of potencies ED_{50_1}/ED_{50_2}.

The factor of the ratio may be used as already described to obtain the limits of the ratio for 19/20 probability. If this is done, it is evident that if the lower limit is greater than 1.0 the ratio is significant. However, the lower limit can exceed 1.0 only if the value of the ratio exceeds that of the factor.

Therefore, two curves may be considered parallel if S.R., the slope function ratio, *does not exceed* its factor, $f_{S.R.}$; and two potencies may be considered significantly different if P.R., the potency ratio, *exceeds* its factor $f_{P.R.}$.

4. *Confidence limits for 19/20 probability of doses other than the median effective dose, ED_{50}.* A satisfactory approximation to the limits of errors of ED_Y (where Y is a response other than 50 per cent) can be derived from the expression for the (standard error)^2 of a log dose whose probit response y deviates from the mean probit, $\bar{y}$ of the experiment. This expression which Bliss (1) gives can be written as:

$$S.E._{ED_y}^2 = (S.E._b s^2)^2(y - \bar{y})^2 + V_a s^2 \qquad (12)$$

It has been shown that:

$$S.E._b s^2 = S.E._a \qquad (4)$$

Furthermore, the first of the approximations of the Litchfield and Fertig method was derived as:

$$V_a s^2 \sim S.E._{\log ED_{50}}^2 \sim \frac{2s^2}{N'} \qquad (1A)$$

By substitution of (4), (1A), and the approximation:

$$(y - \bar{y}) \sim (y - 5.0) = X$$

equation (12) becomes:

$$SE_{\log ED_y}^2 = [S.E._a)(X)]^2 + S.E._{\log ED_{50}}^2$$

which is the same as:

$$(\log f_{ED_y})^2 = (\log [(f_S)^X])^2 + (\log f_{ED_{50}})^2$$

the antilogarithm of the root of this expression is in a form suitable for use in the revised method dealing with arithmetic doses and per cent responses, namely:

$$f_{ED_y} = \text{antilog } \sqrt{(\log [f_S^X])^2 + (\log f_{ED_{50}})^2} \qquad (13)$$

since Nomograph No. 4 solves expressions of this type. The value of X can be read from any table relating deviations and areas of the normal curve (17). Thus, 16 and 84 per cent effect both depart from 50 by 34. The area, 34 per cent, corresponds to a deviation X, of 1.00 in the table cited above. For convenience certain commonly used values of X are given in table 3.

In certain cases one may desire to adopt confidence limits for probabilities other than 19/20. This may be done easily by using the proper multiplier for the exponents of S and A before reading $f_{ED_{50}}$ and f_s from Nomograph No. 2. For probabilities commonly used these multipliers are listed below:

For p of:	*Multiply exponent by:*
0.32 (2/3 odds)	0.51
0.10 (9/10 odds)	0.84
0.05 (19/20 odds)	1.00
0.02 (49/50 odds)	1.19
0.01 (99/100 odds)	1.31
0.001 (999/1000 odds)	1.63

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RESPIRATORY STIMULANTS IN ACUTE COCAINE POISONING IN RABBITS

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The development of a barbital treatment of acute cocaine poisoning in laboratory animals by Tatum and his co-workers (1, 2, 3) has been the most significant contribution to this subject in recent years and has already given promise of its adaptation to acute poisoning in man (4). The convulsions are prevented or relieved and the central nervous system, thus being spared the injury and exhaustion of the convulsions *per se*, is apparently able to withstand a considerably larger dose of cocaine before death results. They were able to raise the minimal lethal dose about one third in the rabbit, about double in the dog, and about three times in the monkey.

Death when convulsions are prevented seems to be due to the action of cocaine chiefly on the respiratory center. In the rabbit, at least, respiratory failure usually precedes cardiac failure and rabbits can survive approximately three times the average fatal dose if adequate artificial respiration is maintained (2). Barbital, however, is relatively ineffective in raising the lethal dose even though the convulsions are readily prevented or relieved. It was thought, therefore, that respiratory stimulation might have a beneficial effect in the rabbit poisoned with cocaine and that the efficiency of respiratory stimulants could be studied in this way. It has been pointed out by a number of workers, particularly by Loevenhardt, Malone, and Martin in their studies on respiratory stimulants in conditions of increased intracranial pressure (5) that the relative value of such stimulants can not be gauged by a single method as some respiratory stimulants are beneficial during one type of respiratory depression but not in another.

On the other hand, if cocaine kills by virtue of an over-excitability of the respiratory center, the use of respiratory stimulants might be harmful and there might result an increased susceptibility to cocaine in animals under the influence of such stimulants.

METHODS

One hundred and forty-eight adult rabbits were used. The cocaine was administered subcutaneously as the 10 per cent solution of the hydrochloride. The hypnotic was administered either orally, subcutaneously, intraperitoneally, or intravenously. The hypnotics used were barbital, barbital-sodium, and the barbital-paraldehyde mixture of Tatum. As a rule, the hypnotics when not given intravenously, were given before the cocaine, the latter being given as soon as evidences of effect were present, or within a half hour if no change in the animal's behavior was noted. The respiratory stimulants used were atropine sulphate, strychnine sulphate, alpha-lobeline, homocamfin, and in a few experiments, caffeine citrate. These were all given subcutaneously usually within five minutes after the cocaine, occasionally at the same time or prior thereto. Atropine sulphate was used in amounts of 0.5 to 1 mgm. per kilogram, strychnine sulphate 0.2 to 0.4 mgm. per kilogram, alpha-lobeline, 1 to 10 mgm. per kilogram, homocamfin 25 to 150 mgm. per kilogram, and caffeine citrate 10 to 20 mgm. per kilogram. The rabbits were kept under observation for several days and were not credited as surviving unless they lived three days or more after the cocaine administration.

RESULTS

The results are shown in tables 1 to 9, which require no explanation. Quite early in this work, it became apparent that there is a rather wide variation in the resistance of rabbits to cocaine and that to attempt to state what the fatal dose per kilogram is, and how this varies with treatment, is confusing. For example, some untreated rabbits will die from 100 mgm. per kilogram, and some will survive from 200 mgm. per kilogram. We believe that the results are much more suitable for compari-

TABLE 1

Cocaine poisoning in untreated rabbits

Rabbits T1, T2, A1, and A3, from Tatum, Atkinson, and Collins.

RABBITS	COCAINE*	RECOVERED	DIED
14, 3	50	14, 3	
A1, A3	85	A3	A1
T1, T2, 50, 127, 142	100	T1, 127	T2, 50, 142
115, 116, 117, 130, 136, 137, 138, 139	150	116, 117, 130, 136, 139	115, 137, 138
101, 103, 104, 118, 131, 132, 133, 134, 135	200	101, 135	103, 104, 118, 131, 132, 132, 133, 134
140	250		140

* Cocaine as Cocaine Hydrochloride in milligrams per kilogram.

TABLE 2

Cocaine poisoning in barbitalised rabbits

T3, T4, T6, T7, T8, T9, T10, T11, T12, and T13, from Tatum, Atkinson, and Collins.

RABBITS	COCAINE	RECOVERED	DIED
T3, T4, 49, 141	100	T3, T4, 49, 141	
T6, T7, T8, T9, T10, T11	150	T8, T9, T10, T11	T6, T7
T12, 38, 39, 41, 52, 57, 59, 61, 62, 63, 68	200	57, 61	T12, 38, 39, 41, 52, 59, 62, 63, 68
T13	250		T13

TABLE 3

Atropine sulphate in barbitalized rabbits poisoned with cocaine

RABBITS	COCAINE	RECOVERED	DIED
143	150	143	
23, 25	175	23, 25	
30, 64, 55, 65, 66, 69, 51, 67, 60	200	30, 64, 55, 65, 66, 69, 51	67, 60
46, 28, 31, 113	250		46, 28, 31, 113
37, 48, 34, 29, 45, 53, 43	300	37, 48	34, 29, 45, 53, 43
40	325		40
44, 47, 112	350	44	47, 112
27	400		27

son and afford less basis for misunderstanding when expressed as mortality rates per given dose. We have, therefore, prepared in table 10, a summary of the previous tables giving the mortality percentage at a given dose of cocaine. If one were to base such

TABLE 4

Strychnine sulphate in barbitalized rabbits poisoned with cocaine

RABBITS	COCAINE	RECOVERED	DIED
128	150		128
81, 80, 79, 74, 72, 71, 70, 73, 75, 76, 78, 77, 129	200	81, 80, 79, 74, 72, 71, 129	70, 73, 75, 76, 78, 77
82	350		82

TABLE 5

Homocamfin in barbitalized rabbits poisoned with cocaine

RABBITS	COCAINE	RECOVERED	DIED
124, 144, 145	150	124, 144, 145	
92, 93, 94, 95, 96, 125, 126, 147, 148	200	92, 94, 95, 146	93, 96, 125
	250	126, 148	147

TABLE 6

Alpha lobeline in barbitalized rabbits poisoned with cocaine

RABBITS	COCAINE	RECOVERED	DIED
5, 4	60	5, 4	
8	130		8
1, 6, 10, 11	150		1, 6, 10, 11
7	160		7
2, 83, 85, 86, 89, 84, 87, 88, 90	200	83, 85, 86, 89	2, 84, 87, 88, 90
9	260		9
42	300		42
91	350		91

a mortality percentage at a given dose upon only the animals to which that dose were given, discordant results would appear unless a very large number of animals were available. For example, from table 1, it is seen that of 5 rabbits given 100 mgm. of cocaine hydrochloride per kilogram, 3 died with a mortality for

these 5 of 60 per cent, while of 8 rabbits given 150 mgm. per kilogram, only 3 died with a mortality for the 8 of 37 per cent. It seems to us obvious that a rabbit that died from 100 mgm. would have died from any higher dose and one that survived from 150 mgm. per kilogram would have survived from any lesser dose.

TABLE 7

Caffeine citrate in barbitalized rabbits poisoned with cocaine

RABBITS	COCAINE	RECOVERED	DIED
13, 15, 16, 17	150	13, 15	16, 17

TABLE 8

Atropine sulphate plus alpha lobeline in barbitalized rabbits poisoned with cocaine

RABBITS	COCAINE	RECOVERED	DIED
12, 18, 19, 20, 21	150	12, 18, 19, 20, 21	
22, 24, 26	175	22, 24, 26	
120, 121	200	120, 121	
32	225	32	
33, 122	250	33, 122	
35	270	35	
54, 36, 123	300	54	36, 123

TABLE 9

Atropine sulphate in rabbits poisoned with cocaine (no barbital)

RABBITS	COCAINE	RECOVERED	DIED
97, 108	100	97, 108	
98, 100, 107, 109, 110	150	98, 100, 109, 110	107
102, 99, 58, 106, 111, 119	200	102, 99, 111, 119	58, 106
105, 114	250	105	114

This rationale has been used therefore in calculating the mortality percentages in table 10. We have included under each percentage-figure the number of animals upon which the calculation is based, as the figure is correspondingly more significant, the larger the number of animals in the group.

DISCUSSION

Barbital was found effective in all instances where given in adequate dosage in preventing or relieving the convulsions due to cocaine. It was not, however, very effective in decreasing the mortality percentage, the mortality of barbitalized rabbits from

TABLE 10

Summary. Mortality percentage in acute cocaine poisoning in rabbits

The numbers in parentheses represent the number of animals from which the percentage is calculated.

COCAINE	(1) UNTREATED	(2) BARBITAL	(3) BARBITAL + ATROPINE	(4) BARBITAL + STRYCHNINE	(5) BARBITAL + HOMOCAMPIN	(6) BARBITAL + ALPHA-LOBELINE	(7) BARBITAL + CAFFEINE	(8) BARBITAL + ATROPINE + ALPHA-LOBELINE	(9) ATROPINE
mgm. per kgm.									
50	0% (12)	0% (8)	0% (13)	0% (7)	0% (9)	0% (6)	0% (2)	0% (15)	0% (11)
100	31% (13)	0% (8)	0% (13)	0% (7)	0% (9)	0% (4)	0% (2)	0% (15)	0% (11)
150	50% (14)	33% (6)	0% (13)	12% (8)	0% (9)	55% (9)	50% (4)	0% (15)	10% (10)
200	88% (16)	85% (13)	17% (12)	50% (14)	33% (9)	73% (15)	100% (2)	0% (7)	38% (8)
250	100% (15)	100% (12)	67% (9)	100% (7)	67% (6)	100% (11)		0% (4)	80% (5)
300			80% (14)		100% (4)			67% (3)	100% (4)
350			94% (15)					100% (2)	

100 mgm. of cocaine per kilogram being 0 per cent as against 31 per cent for the untreated, and 33 per cent at 150 mgm. as against 50 per cent for the untreated. Approximately 100 mgm. of barbital per kilogram was the minimum effective dose. This was

also the optimum dose in our experience, although as high as 200 mgm. per kilogram can be given without any danger of too great depression. Atropine, strychnine, and homocamfin, when given to barbitalized rabbits decreased the mortality to cocaine, atropine being the most efficient. Alpha-lobeline was relatively ineffective alone, although when used in conjunction with atropine, it seemed to be of some benefit. Caffeine was not found of any benefit, although the number of animals used is not enough for conclusions. Because of the effectiveness of atropine in the barbitalized animals it was thought best to try its effect on rabbits in which no barbital was given. The results as shown in table 9, and in the summary table, column 9, indicate that it has a marked effect in saving animals poisoned with cocaine; and this in spite of the fact that all animals showed convulsions. These findings would indicate that the convulsions *per se* are not of such serious significance, at least in the rabbit, as is the respiratory depression for our results with atropine alone are better than with barbital alone. The beneficial effects of these various drugs is attributed to a stimulation of the respiratory center and therefore a physiological antagonism to the depressant effects produced by cocaine. It seems difficult to correlate their beneficial effects on any other basis. Seevers and Tatum (6) have shown that vagotomy is without effect so that in the case of atropine there is no reason to believe that its effect is on peripheral mechanisms.

CONCLUSIONS

1. The beneficial effect of barbital on cocaine convulsions in the rabbit as reported by Tatum *et al.* is confirmed.

2. Respiratory stimulants are of value in the treatment of acute cocaine poisoning in the rabbit, atropine being the most effective of the ones tried. The best results were obtained where barbital and respiratory stimulation were used together.

3. These results would indicate that death does not occur from an over excitability of the respiratory center in cocaine poisoning but from a true depression which is counteracted in part by stimulation.

FICHE TECHNIQUE N° 6

J. Pharmacol. (Paris), 1970, 1, 3, 407-414.

Supplément au n° 3

Souris
Toxicité aiguë

DÉTERMINATION DE LA DL 50 CHEZ LA SOURIS

(Méthode de Litchfield et Wilcoxon) (1)

Principe.

Parmi les diverses méthodes d'évaluation de la toxicité d'un médicament, l'appréciation de la dose léthale 50 (DL 50) consiste à déterminer la dose de ce médicament qui est mortelle pour 50 % des animaux mis en expérience.

Les souris sont réparties en lots aussi semblables que possible. Les animaux de chaque lot reçoivent une même dose de produit à étudier et les doses diffèrent d'un lot à l'autre. L'on apprécie le résultat selon un critère de tout ou rien (mortalité ou survie) dans chacun des lots.

Ce qu'il faut.

- balance pèse-souris,
- matériel classique d'administration, de marquage, de dissection,
- papier logarithme-probits n° 543 (Compagnie Française des Diagrammes, 24, boulevard d'Inkermann, 92 - Neuilly),
- règle transparente,
- table des carrés,
- tableaux I et II, abaques I, II et III de LITCHFIELD et WILCOXON (ci-joints).

Ce qu'il faut faire.

I. — NOTER SOIGNEUSEMENT LES CRITÈRES INHÉRENTS AUX SOURIS :

- souche ;
- sexe ;
- poids : 18 à 22 g ;
- état physiologique : sauf cas très spéciaux, on évitera les femelles gravides ;
- état sanitaire : on aura avantage à opérer sur des animaux E.O.P.S. exempts d'organismes pathogènes spécifiques ;
- jeûne préalable, éventuellement.

(1) Fiche rédigée par Charlotte DUPONT (maître-assistant), *Laboratoire de Pharmacodynamie, Faculté de Pharmacie, 4, avenue de l'Observatoire, F. 75 - Paris-06.*

FICHE TECHNIQUE

TABLEAU I. — *Valeurs corrigées des effets 0 ou 100 % correspondant aux valeurs attendues*
(*N. B.* Ces valeurs sont dérivées des probits max. et min. corrigés de Bliss)

Attendus	0	1	2	3	4	5	6	7	8	9
0	—	0,3	0,7	1,0	1,3	1,6	2,0	2,3	2,6	2,9
10	3,2	3,5	3,8	4,1	4,4	4,7	4,9	5,2	5,5	5,7
20	6,0	6,2	6,5	6,7	7,0	7,2	7,4	7,6	7,8	8,1
30	8,3	8,4	8,6	8,8	9,0	9,2	9,3	9,4	9,6	9,8
40	9,9	10,0	10,1	10,2	10,3	10,3	10,4	10,4	10,4	10,5
50	—	89,5	89,6	89,6	89,6	89,7	89,7	89,8	89,9	90,0
60	90,1	90,2	90,4	90,5	90,7	90,8	91,0	91,2	91,4	91,6
70	91,7	91,9	92,2	92,4	92,6	92,8	93,0	93,3	93,5	93,8
80	94,0	94,3	94,5	94,8	95,1	95,3	95,6	95,9	96,2	96,5
90	96,8	97,1	97,4	97,7	98,0	98,4	98,7	99,0	99,3	99,7

TABLEAU II. — *Valeurs de t et de χ^2 pour $p = 0.05$*

Degrés de liberté	t	χ^2
1	12,7	3,84
2	4,3	5,99
3	3,18	7,82
4	2,78	9,49
5	2,57	11,1
6	2,45	12,6
7	2,36	14,1
8	2,31	15,5
9	2,26	16,9
10	2,23	18,3

II. — SIGNALER LES CONDITIONS D'ENVIRONNEMENT :

— température ambiante ;

— nombre d'animaux par boîte.

III. — PRÉCISER LES RENSEIGNEMENTS RELATIFS A L'ADMINISTRATION :

— voie d'administration ;

— volume administré : il doit être constant pour tous les lots, et au maximum de 0,5 ml par 20 g de poids corporel ;

— vitesse d'injection pour une administration parentérale ;

— concentration en principe actif ;

— nature du véhicule : s'il n'est pas aqueux, il faudra administrer le véhicule seul à des souris témoins ;

— doses administrées : les choisir en progression géométrique (pour que leurs logarithmes soient en progression arithmétique) ;

— nombre de souris par lot : au minimum 20.

DÉTERMINATION DE LA DL 50 CHEZ LA SOURIS

N. B. — 1° Après un repérage rapide, il sera nécessaire d'utiliser un plus grand nombre d'animaux pour les doses médianes afin d'augmenter la précision de la mesure. Ainsi un pourcentage de 40 %, déterminé par la mort de 4 animaux sur 10 comporte, pour une probabilité de 95 %, des limites de confiance de 12 à 74 % ! Il est donc nécessaire de recourir à des lots d'au moins 20 animaux.

N. B. — 2° S'il n'est pas possible de procéder à l'administration de la solution à tous les animaux au cours de la même séance, il est recommandé de traiter une partie des animaux de chacun des lots étudiés, et de compléter le lendemain, afin de minimiser l'influence des conditions opératoires, plutôt que d'étudier l'effet des diverses doses à des jours différents.

IV. — INDIQUER LES RÉSULTATS DE L'ESSAI :

- — symptômes observés ;
- — délai d'appréciation de la mortalité globale (ce délai devant être au minimum de sept jours et augmenté en cas de troubles) ;
- — résultats de l'autopsie pratiquée immédiatement après le décès (indispensable en cas de mort rapide après intubation) ;
- — tableau des pourcentages de mortalité suivant les doses.

Résultats.

description of regression line

Le procédé de BEHRENS et KÄRBER repose sur des bases théoriques discutables ; celui de MILLER et TAINTER peut suffire si l'on ne recherche qu'un résultat approximatif ; la méthode de BLISS est très rigoureuse mais très longue. Nous décrivons ci-après la méthode de LITCHFIELD et WILCOXON compte tenu de la rapidité de son exécution et de sa précision.

- — Etablir un tableau des résultats obtenus et des pourcentages de mortalité pour chaque dose.
- — Reporter les résultats sur un papier spécial logarithme-probit, sauf 0 et 100 % ; porter directement en abscisses les diverses doses administrées, et en ordonnées les pourcentages de mortalité correspondants.
- — Tracer la *droite de régression provisoire* qui doit s'adapter le plus possible aux points expérimentaux, une importance particulière étant accordée aux points situés dans l'intervalle 40 % à 60 %.
- — Lire et noter sur la droite tracée les *pourcentages « attendus »* en regard des doses administrées.
- — Calculer à l'aide du tableau I à partir des pourcentages attendus les valeurs pour 0 % et 100 % de mortalité. Vérifier que la droite tracée passe bien par ces points, sinon recommencer.
- — Faire la différence : pourcentage observé-pourcentage attendu, pour chacune des doses. A l'aide de l'abaque I, tracer une droite entre les valeurs du pourcentage attendu (échelle de gauche) et de la différence calculée ci-dessus (échelle centrale), et la prolonger jusqu'à la colonne de droite. Lire ainsi la *« contribution au χ^2 »*.

exposant (échelle de droite). Le point d'intersection avec l'échelle centrale donne directement le facteur de correction *f* DL 50.

— Pour le seuil de probabilité P = 0,05, les limites de la DL 50 sont les suivantes :

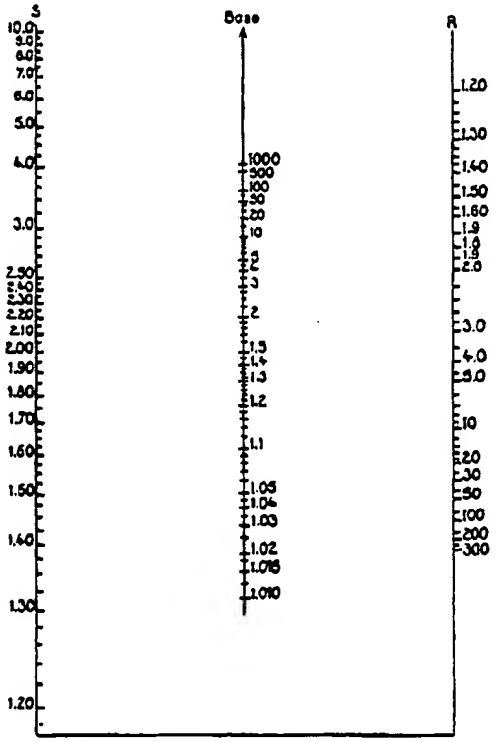
$$\text{Limite supérieure} = \text{DL } 50 . f \text{ DL } 50$$

$$\text{Limite inférieure} = \frac{\text{DL } 50}{f \text{ DL } 50}$$

LIMITES DE CONFIANCE DE LA PENTE DE LA DROITE S.

— Calculer le *rapport R de la dose la plus forte à la dose la plus faible.*

— A l'aide de l'abaque III, tracer une droite entre les valeurs de S (échelle de gauche) et de R (échelle de droite). L'intersection de cette droite avec l'échelle centrale donne la base A.



ABAQUE III.

DÉTERMINATION DE LA DL 50 CHEZ LA SOURIS

LIMITES DE CONFIANCE DE LA DL 50.

— Lire sur le graphique les *doses correspondant aux effets 16, 50 et 84 p. 100* (DL 16, DL 50 et DL 84).

— Calculer la *pente S de la droite :*

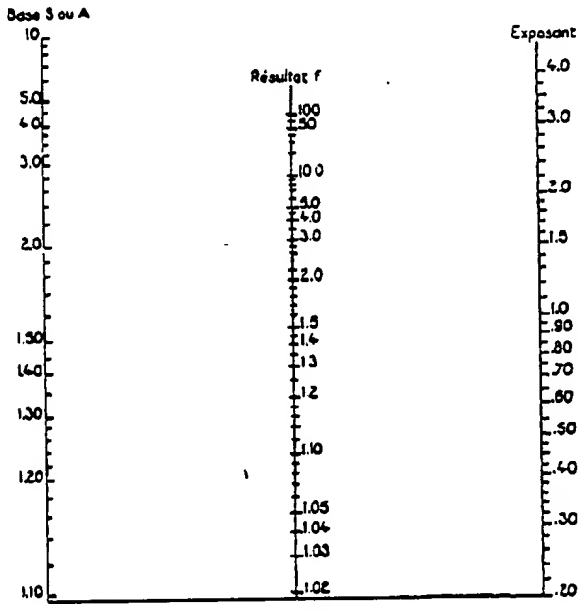
$$S = \frac{\frac{DL\,84}{DL\,50} + \frac{DL\,50}{DL\,16}}{2}$$

— Chercher, d'après les données, le *nombre total N' d'animaux* qui ont été utilisés *pour obtenir les points situés entre DL 16 et DL 84* (attendus).

— Calculer $\sqrt{N'}$, puis $\frac{2,77}{\sqrt{N'}}$, exposant de S dans l'expression du facteur de correction *f* DL 50 :

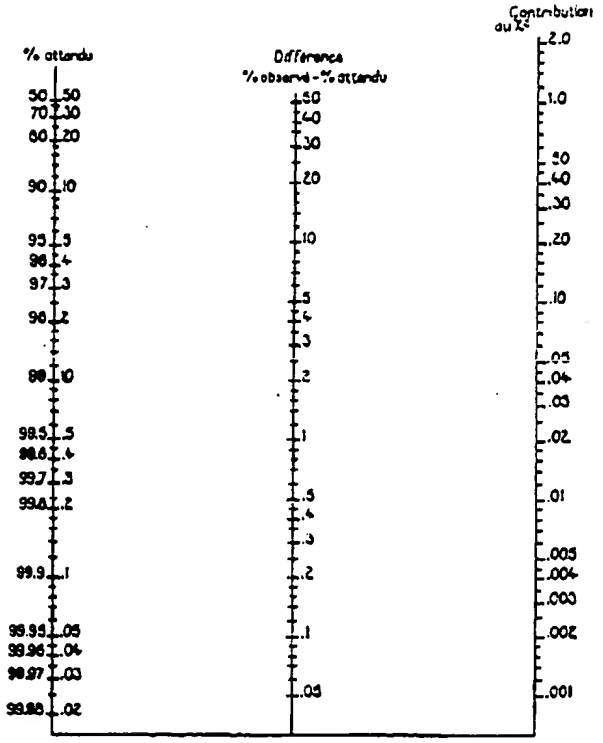
$$f\,DL\,50 = S^{\frac{2,77}{\sqrt{N'}}}$$

— Ce facteur peut être lu directement sur l'abaque II en procédant de la manière suivante : tracer une droite reliant la valeur de S (échelle de gauche) et celle de son



ABAQUE II.

FICHE TECHNIQUE



ABAQUE I.

— *Faire la somme des diverses contributions au* χ^2 : Σ cont. χ^2. Déterminer le χ^2 de la droite en multipliant cette somme par le nombre moyen $\frac{N}{K}$ d'animaux par dose.

K = Nombre total de doses.

N = Nombre total d'animaux.

$\frac{N}{K}$ = Nombre moyen d'animaux par dose.

$n = K - 2$ = Nombre de degrés de liberté.

$$\chi^2 = \Sigma \text{ cont. } \chi^2 . \frac{N}{K}$$

— Chercher la valeur de χ^2 dans le tableau II pour le seuil de probabilité $p = 0,05$ et pour ce nombre n de degrés de liberté. Si la valeur du χ^2 expérimental est inférieure à celle du tableau, la droite est bien tracée. Si elle est supérieure, la droite est mal tracée. La tracer de nouveau et recommencer les opérations ci-dessus.

— Connaissant K et $\sqrt{N'}$, calculés précédemment, déterminer le quotient $\frac{10\,(K-1)}{K\sqrt{N'}}$, exposant de A dans l'expression fS, facteur de correction de la pente S de la droite :

$$fS = A^{\frac{10\,(K-1)}{K\sqrt{N'}}}$$

— Au moyen de l'abaque II, déterminer le *résultat* fS connaissant A et son exposant.

— Pour le seuil de probabilité $p = 0{,}05$, la limite supérieure de S est $S.fS$ et la limite inférieure $\frac{S}{fS}$.

N. B. 1. — Si le test du χ^2 a montré une hétérogénéité, il faut utiliser le procédé de calcul comportant le coefficient t du tableau II.

$$f\,DL\,50 = S^{1{,}4\,t\sqrt{\frac{\chi^2}{n\,N'}}}$$

$$fS = A^{\frac{5{,}1\,t\,(K-1)\,\sqrt{\frac{\chi^2}{n\,N'}}}{K}}$$

N. B. 2. — Cette méthode est également applicable au calcul de la dose efficace 50 % (DE 50).

Exemple : toxicité du chlorhydrate de cocaïne chez la Souris (Finney).

Dose (mg/kg)	Nombre d'animaux	Nombre de morts	Pourcentage observé	% attendu	Différence entre % observé et % attendu	Contribution au χ^2
0,015	20	0	0 (0,94)	2,8	1,96	0,014
0,020	69	11	16	18,5	2,5	0,0045
0,025	95	50	53	46	7	0,020
0,030	78	61	78	71	7	0,023
0,035	44	37	84	86,5	2,5	0,0055
0,040	20	20	100 (98,2)	94,5	3,7	0,027
	N = 236				Σ cont. χ^2 =	0,094

$K = 6$ $\quad n = 6 - 2 = 4$ degrés de liberté.

$\chi^2 = 0{,}094.\ \frac{236}{6} = 3{,}7.$

χ^2 table pour 4 D.L. = 9,49, donc la droite est correctement tracée.

FICHE TECHNIQUE

DL 16 = 0,0195 DL 50 = 0,0255 DL 84 = 0,0340

$$S = \frac{\frac{0.0340}{0.0255} + \frac{0,0255}{0.0195}}{2} = 1,32$$

$N' = 69 + 95 + 78 = 242$ $\sqrt{N'} = 15.55$ $\frac{2,77}{\sqrt{N'}} = 0,178$

$f\,DL\,50 = 1,32^{0,178} = 1,05$

$DL\,50.\ f\,DL\,50 = 0,0255.\ 1,05 = 0,0268$ $\frac{DL\,50}{f\,DL\,50} = \frac{0,0255}{0,05} = 0,0243$

$0,0243 < DL\,50 < 0,0268$ mg/kg

La méthode de Bliss pour les mêmes chiffres aboutissait (in Valette [5]) à :

$0,0241 < DL\,50 < 0,0261$ mg/kg

$R = \frac{0,040}{0,015} = 2.66$ $A = 1,09$

$f\,S = 1,09^{\frac{10\,(3)}{6\,(15,53)}} = 1,09^{0,503} = 1,047$

$S.\ f\,S = 1,32.\ 1,047 = 1,38$

$\frac{S}{f\,S} = \frac{1,32}{1,047} = 1,26$

$1,26 < S < 1,38$

Ces limites sont utiles à connaître pour déterminer l'intervalle de confiance des points autres que la DL 50.

Conclusion.

L'estimation chez la Souris de la DL 50 avec ses limites de confiance selon la méthode de Litchfield et Wilcoxon permet de déterminer avec précision la toxicité aiguë d'un médicament. Cette DL 50 servira de point de repère pour l'étude des propriétés pharmacodynamiques du médicament.

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is impossible and resigns itself to the experimental conditions. This hypothesis receives support from results presented below which indicate that immobility is reduced by different treatments known to be therapeutic in depression including three drugs, iprindole, mianserin and viloxazine which although clinically active[1-3] show little or no 'antidepressant' activity in the usual animal tests[1-6].

Naive male Sprague–Dawley (Charles River) rats weighing between 160 and 180 g are plunged individually into a vertical plexiglass cylinder (height 40 cm; diameter 18 cm) containing 15 cm of water maintained at 25 °C. After 15 min in the cylinder they are removed and allowed to dry for 15 min in a heated enclosure (32 °C) before being returned to their individual cages. This treatment produces long periods of immobility in the water (10–12 min total duration) and the rats on removal are mildly hypothermic (−3 °C) and are hypoactive for periods up to 30 min. 24 h later the rats are replaced in the cylinder and the total duration of immobility is measured during a 5-min test. Rats submitted to this procedure will remain immobile for 75% of the duration of the test (see Table 1) thus providing a suitable baseline for measuring the effects of drugs which decrease or even increase immobility.

Drugs and doses investigated are shown in Table 1. To optimise the pharmacological effect[7] and at the same time to imitate more closely clinical usage, we administered the drugs in a series of three intraperitoneal injections 24, 5 and 1 h before testing on day 2, the first injection being given immediately before replacing the animals in their cages on day 1. Electroconvulsive shock (ECS; 30 mA, 50 Hz, 1 s) was delivered through ear-clip electrodes according to the same time schedule as for drug injections.

Table 1 shows that all the antidepressants tested as well as ECS significantly reduced immobility. With the tricyclic compounds, particularly amitriptyline, this 'antidepressive' effect was accompanied by lowered muscle tonus and diminished motor activity outside the test situation suggesting sedation. Biphasic effects were observed with mianserin and iprindole: a reduction in immobility at lower doses followed at higher doses by a return to control levels and a marked loss of muscle tonus. Viloxazine decreased immobility without apparent sedative effects. The monoamine oxidase inhibitor, nialamide, reduced immobility but induced small repetitive body movements which were not observed with the tricyclic antidepressants; loss of muscle tonus was noted at 100 mg kg^{-1}. The two psychostimulants (+)-amphetamine and caffeine also reduced immobility but in contrast to the tricyclic compounds induced a marked hyperactivity; with (+)-amphetamine stereotyped head movements were observed at 1.5 and 3 mg kg^{-1}. Neither chlordiazepoxide nor diazepam affected the duration of immobility even at doses which produced noticeable ataxia. In contrast, both chlorpromazine and the reserpine-like compound RO4-1284 increased mobility; with RO4-1284 a ceiling effect was already observed at 1 mg kg^{-1}, a non-cataleptic dose.

We conclude from the results obtained that the immobility induced in these experiments reflects a state of lowered mood in the rat. Immobility was reduced by antidepressant drugs and ECS, was unaffected by anxiolytics, and was increased by drugs known to be capable of inducing depressive states in man[8,9]. The positive findings obtained with amphetamine and caffeine do not stand in contradiction to this conclusion as both compounds do possess some clinical antidepressant activity[10] and besides, the effects observed could be distinguished qualitatively from those of the non-stimulant antidepressants.

The results obtained with mianserin deserve comment because this compound, although clinically active[2], possesses a profile of pharmacological and biochemical activity which is almost the mirror image of that expected of a classical tricyclic antidepressant: it potentiates the effects of reserpine and antagonises the effects of amphetamine[11]. It does not inhibit the re-uptake of monoamines[12] and is moreover a potent blocker of central 5-hydroxytryptamine receptors[13]. Although providing no explanation for the mechanisms of action of mianserin, it is noteworthy that the present procedure, which is based on behavioural rather than biochemical concepts, is the first animal test model which would predict an antidepressant action for this compound. Furthermore the bimodal effect observed strikingly parallels clinical observations showing an optimal antidepressant effect at intermediate doses[14].

These positive findings, together with those obtained with the two other atypical compounds, iprindole and viloxazine, raise the intriguing possibility that the method described here might be capable of discovering new types of antidepressant agents hitherto undetectable using classical screening tests.

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Benzodiazepine receptors in rat brain

High affinity binding of tritium labelled morphine and morphine-like drugs to membranes in brain homogenates[1-3] was a decisive advance in the characterisation of opiate receptors and the discovery of enkephalines and endorphines. We report here experiments which suggest that another important group of psychoactive drugs, the benzodiazepines, bind to specific receptors on the membranes of rat brain cells. This suggests that there may be an unknown endogenous neurotransmitter which is the natural ligand for the benzodiazepine receptor. The binding sites are distributed unevenly through the brain, and displacement potencies of benzodiazepines correlate with pharmacological effects predictive of anxiolytic activity.

Whole forebrains (excluding cerebellum and pons-medulla) of 150 g Wistar rats were homogenised gently in 20 volumes of ice-cold 0.32 M sucrose, centrifuged at 1,000*g* for 10 min and recentrifuged at 30,000*g* to give a crude P_2-synaptosomal fraction. The P_2-fraction was rehomogenised in an equal volume of hypotonic, 50 mM, Tris-HCl (*p*H 7.5). The binding assay consisted of 500 µl test drug solution and with ^{3}H-diazepam as the radioactive ligand, usually at 1.6 nM (^{3}H-diazepam (*N*-methyl-^{3}H) 14, 4 Ci mmol^{-1}; provided by Dr Willy Haefely, Hoffmann-La Roche). The mixture was preincubated for 5 min at 37 °C before addition of ^{3}H-diazepam followed by a 15 min additional incubation. The

samples were cooled for 30 min in an ice bath and membranes with bound ^{3}H-diazepam were isolated on Whatman GF/C glassfibre filters. At the end of the incubation 10 ml of iced buffer (50 mM Tris-HCl, *p*H 7.4 was added, immediately before filtering, and the filter was washed with an additional 10 ml iced buffer and bound diazepam was estimated by conventional scintillation counting.

The amount of nonspecific binding, defined as binding in the presence of excess unlabelled ligand, was very low. Addition of diazepam at 3 μM to the binding assays displaced 91–93% of the total ^{3}H-diazepam binding. Specific ^{3}H-diazepam binding was saturable (Fig. 1) and maximal binding was obtained with about 50 nM ^{3}H-diazepam. A Scatchard analysis of the results in Fig. 1 yielded a single straight line in the concentration range below 50 nM, indicating the presence of a single binding site. The affinity constant, K_D (concentration of ^{3}H-diazepam giving half-maximal binding) was 2.6 nM and the total number of binding sites was 18 pmol per g original tissue. Hill plots (not shown) had a slope of 1.0–1.1 indicating absence of cooperativity.

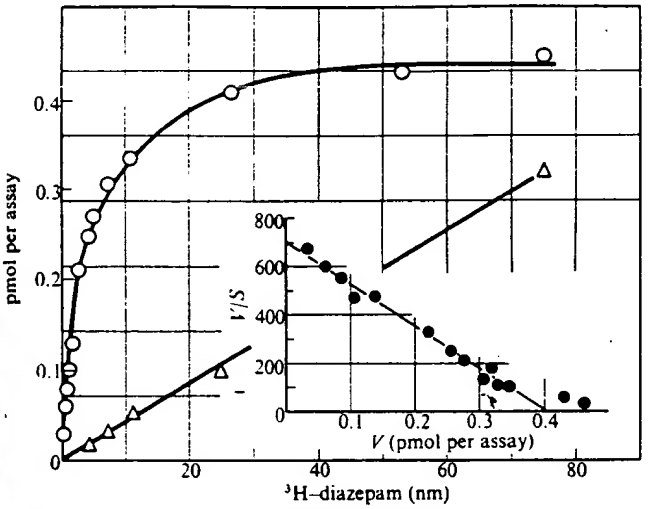

Fig. 1 Binding of ^{3}H-diazepam to membranes from 25 mg rat forebrain. ^{3}H-diazepam, 0.24–75 nM was incubated in triplicate with or without excess cold diazepam. Specific binding (○) is the total binding minus the binding which is not displaced by excess (3 μM) diazepam (●). Insert shows the same data transformed to Scatchard plots (*V*, specific binding in pmol per 25 mg tissue; *S*, ^{3}H-diazepam concentration in pM). The Scatchard plot indicates one binding site with affinity constant, K_D = 2.6 nM (reciprocal of the slope value) and maximal binding of 18 pmol per g original tissue (intercept on abscissae). The experiment was repeated, giving closely similar results. Counting efficiency was 44%.

Measurement of specific ^{3}H-diazepam binding (at 1.6 nM) in 10 rat brain regions showed an uneven regional distribution. The greatest binding was found in the frontal and occipital cortex where the binding was three to fourfold higher than the lowest binding in the pons-medulla. The hippocampus, which may be an important site of action for benzodiazepines in the limbic system[4], had intermediate binding levels.

The ^{3}H-diazepam binding site was highly selective for benzodiazepines. Twenty one benzodiazepines (Table 1) displaced ^{3}H-diazepam with IC_{50} values in the range of 3×10^{-9} to 10^{-4} M while some other 'minor tranquillisers', such as meprobamate, barbiturates and ethanol, were inactive at 0.1 mM. Several presumed neurotransmitters were tested as ^{3}H-diazepam displacers. None of these (acetylcholine, noradrenaline, dopamine, serotonin, GABA, L-glutamate or glycine, all at 0.1 mM) or their antagonists (phenoxybenzamine, propranolol, pimozide, clozapine, methysergide, bicuculline, picrotoxin or strychnine, all at 3×10^{-6} M) exhibited any displacement activity. Various other compounds, including etorphine, nalorphine and muscimol (all at 3×10^{-6} M) were also inactive.

Even though the binding of a compound may be saturable and show remarkable selective displacement properties it may not occur at meaningful recognition sites[5]. The diazepam receptor which we describe here, however, seems to be physiologically significant since it can be correlated to pharmacological activities. Efficacies in several pharmacological tests are available for most of the benzodiazepines used in the present study[6]. Excellent correlations ($P > 0.001$) were observed between ^{3}H-diazepam displacement potency on the one hand and cat muscle relaxant effect, impairment of mouse rotarod performance, inhibition of electric shock induced fighting in mice and antagonism of pentazol induced convulsions in mice, on the other. Weak correlation was found to taming of cynomolgus monkeys ($P = 0.01$, $r = 0.655$). Displacement of ^{3}H-diazepam binding correlates best with the cat muscle relaxant effect (Fig. 2). While the pharmacologial tests listed above are predictive of anxiolytic and anticonvulsant activity we found no correlation with the non-predictive conditioned avoidance tests (either shock increase or escape failures) in the rat.

Table 1 Inhibition of specific ^{3}H-diazepam binding (1.6 nM) to rat brain membranes by benzodiazepines

Compound*	IC_{50}(nM)†
RO 5–4023 (clonazepam)	5
RO 5–4200 (flunitrazepam)	5
Lorazepam	7
RO 5–3027	9.2
RO 5–3590	13.1
RO 5–6901 (flurazepam)	28
RO 5–2807 (diazepam)	34
RO 5–3059 (nitrazepam)	34
RO 5–3350 (bromazepam)	48
RO 6–6616 (chlorazepate)	62
RO 5–2904	76
Oxazepam	80
RO 5–2181	135
RO 5–4528	389
Chlordiazepoxide	1,072
RO 5–5807	4,487
RO 5–3785	5,686
RO 5–4556 (medazepam)	6,217
RO 5–3636	8,270
RO 5–4933	< 30,000
RO 5–4864	163,522

Serial dilutions of benzodiazepines (in duplicate experiments) were added to the high affinity binding assay (see text).
*Chemical structures given in ref. 6.
†IC_{50}, concentration causing 50% inhibition of specific ^{3}H-diazepam binding.

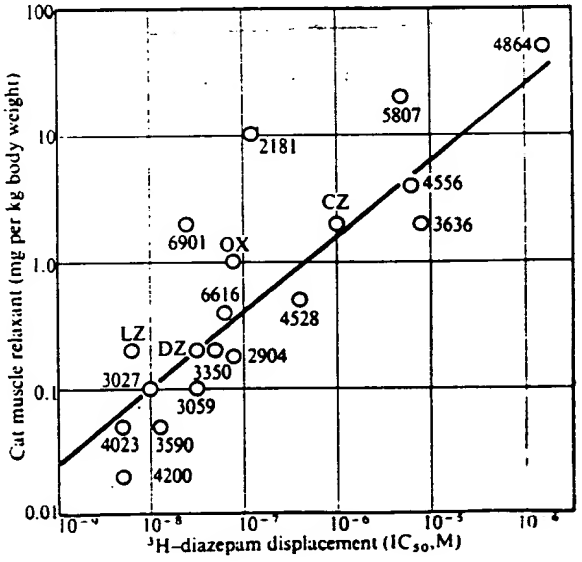

Fig. 2 Correlation between IC_{50} values for ^{3}H-diazepam displacement by 19 benzodiazepines (from Table 1) and cat muscle relaxant (oral dosage) effect. The best curve fit was assessed by linear regression analysis ($r = 0.891$; $P < 0.001$). Numbers on graph indicate RO code number, OX, oxazepam; DZ, diazepam; LZ, lorazepam; CZ, chlordiazepoxide.

In a comprehensive review, Randall *et al.*[6] concluded that the 'actions of benzodiazepines cannot be related in a direct manner to the action of neurotransmitters in the brain'. The proposal that benzodiazepines act on glycine receptors seems now unlikely in view of electrophysiological data[8,9] and our finding that neither glycine nor its antagonist strychnine displace ^{3}H-diazepam binding. Further, the benzodiazepines were about 10,000 times more potent as displacers of ^{3}H-diazepam binding than ^{3}H-strychnine binding. And ^{3}H-diazepam does not apparently bind to GABA-receptors, since GABA itself, its potent agonist muscimol and its antagonists picrotoxin and bicuculline were completely inactive as ^{3}H-diazepam displacers. The failure of benzodiazepines to block ^{3}H-GABA binding to brain membranes[10] further indicates the lack of direct benzodiazepine interactions with GABA receptors. These results, however, are not inconsistent with the proposal that benzodiazepines exert their physiological effects through GABA[11], since such effects may be indirect (for example, benzodiazepines might facilitate GABA release).

Our findings strongly suggest that the brain possesses specific receptors for benzodiazepines which may mediate their pharmacological actions. The exact mechanisms by which benzodiazepines exert their characteristic pharmacological and clinical effects remain unknown. A search for a hitherto undiscovered endogenous transmitter substance, acting on the benzodiazepine receptor therefore seems worthwhile.

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Axotomy causes loss of muscarinic receptors and loss of synaptic contacts in the hypoglossal nucleus

MUSCARINIC receptors in the brain and ileum can be studied by means of the binding of radiolabelled cholinergic antagonists[1,2]. The basic criteria for receptor-specific labelling are that there should be a saturable component of binding and that the binding of the radiolabelled ligand should be inhibited by pharmacologically effective concentrations of drugs which are known to act at muscarinic receptors and not by drugs that act at different receptors. In addition, the saturable binding component should be restricted to tissues known from pharmacological experiments to contain muscarinic receptors. These criteria are satisfied by tritiated propylbenzilylcholine mustard (^{3}H-PrBCM)[3], a potent irreversible muscarinic antagonist the binding of which to rat brain sections can be demonstrated autoradiographically. The evidence which suggests that the bulk of tissue radioactivity is associated with muscarinic receptors is that in autoradiographs the regional variation of autoradiographic grain density parallels the intensity of specific ^{3}H-PrBCM binding observed by *in vitro* assay on brain homogenates from selected regions, and that in the homogenates 65–85% of the bound radioactivity is blocked by pretreatment with the classical muscarinic antagonist, atropine (10^{-6} M). In the forebrain the general features of the distribution of ^{3}H-PrBCM binding to thin sections correspond closely with the findings of Kuhar and Yamamura[4,5] who used the non-covalently bound antagonist ^{3}H-3-quinuclidinylbenzilate. We report here, however, that in the brain stem the hypoglossal nuclei are heavily labelled with ^{3}H-PrBCM. In light microscopic autoradiographs (Fig. 1*a*, left side) the grains are distributed throughout the neuropil of the hypoglossal nucleus. The cytoplasm and nuclei of the large hypoglossal neuronal somata (Fig. 1*b*) are unlabelled. The density of the silver grains falls abruptly at the borders of the hypoglossal neuropil. This clear demarcation of the labelled hypoglossal neuropil is abolished by atropine pretreatment (Fig. 1*c*).

The present results are based on a total of 48 adult female Wistar rats in which the hypoglossal nerve was cut on one side in the neck. In 30 animals the brains were prepared for light microscopic autoradiographic study of receptor binding, and in a further 18 animals the brains were prepared for an electron microscopic study of synapse numbers.

Several studies[6-9] of the effects of axotomy on the hypoglossal nucleus have shown that by 1 week after operation there is a loss of about 50% of the synapses on the hypoglossal motoneurones. If the hypoglossal axons are able to regenerate and re-establish neuromuscular contacts, synapses reappear in the hypoglossal nucleus, indicating that the synaptic 'disjunction' is reversible. We have examined the hypoglossal nucleus at various times after section of the hypoglossal nerve on one side, and the preliminary series of experiments indicated that two changes are detectable within 2 d after axotomy, and seem to have reached completion by 7–10 d after operation.

There was a marked decrease in the intensity of binding of ^{3}H-PrBCM. Since the nerve arises almost exclusively from the hypoglossal nucleus of the same side, and the two nuclei lie immediately adjacent to each other on either side of the midline, the opposite nucleus can be used as a control in the same coronal section. A representative indication of the magnitude of the changes may be seen from the grain counts taken from the sections shown in Fig. 1*a* and *c*. The numbers of silver grains per 10^4 μm^2 of section were 305±5 over the unoperated hypoglossal nucleus, 209±4 over the hypoglossal nucleus of the operated side (6 d after axotomy), and 123±3 over the hypoglossal nucleus of the unoperated side in the adjacent section (Fig. 1*c*) pretreated with atropine. Subtracting the atropine counts, this indicates that section of the hypoglossal nerve had caused a fall in the intensity of labelling of the hypoglossal nucleus of the operated side to 47% of its intensity on the unoperated side.

At the electron microscopic level, we counted the total numbers of synapses per unit area of section. As in the studies of Sumner[7] we found that there was an almost 50% reduction in the total numbers of synapses by 1 week after operation.

If the hypoglossal nerve is able to regenerate and re-establish contact with an appropriate peripheral target tissue, these changes are reversed. The hypoglossal motoneurones re-acquire a normal complement of afferent synapses[6-9] and our observations on the rats with the longer survival times showed that in those cases where the hypoglossal nerve had grown back, binding of ^{3}H-PrBCM in the hypoglossal neuropil had also recovered to normal levels. In some animals, the regenerating